Vasopressin-containing neurons of the suprachiasmatic nuclei inhibit corticosterone release

Andries Kalsbeek, Ruud M. Buijs, Joop J. van Heerikhuize, Moniek Arts and
Tjitske P. van der Woude

Netherlands Institute for Brain Research, Amsterdam (The Netherlands)

(Accepted 24 December 1991)

Key words: Circadian rhythm; Paraventricular nucleus; Brain lesion; Hypothalamus; Central infusion; Vasopressin antagonist

The suprachiasmatic nucleus (SCN) is the major pacemaker in the central nervous system responsible for generating circadian rhythmicity in mammals. Tracer studies show limited projections of the SCN, mainly to the paraventricular nucleus of the thalamus and paraventricular and dorsomedial nuclei of the hypothalamus, suggesting that the latter two areas may be the target areas of the SCN for controlling corticosterone release. The present results show that when infused in the paraventricular/dorsomedial nucleus of the hypothalamus femtomolar concentrations of vasopressin (VP), but not vasoactive intestinal peptide (VIP), are able to suppress elevated levels of corticosterone in SCN-lesioned animals to basal daytime values. On the other hand, infusion of the VP antagonist in the same hypothalamic area induced a sevenfold increase of basal corticosterone levels in intact animals. The SCN origin of this VP input was established in SCN-lesioned animals where no difference between the effect of infusing the antagonist or Ringer could be detected. These results imply that the SCN can influence the daily corticosterone rhythm through its VP-containing projection to the paraventricular/dorsomedial nucleus of the hypothalamus.

INTRODUCTION

The suprachiasmatic nucleus (SCN) is the major pacemaker in the central nervous system responsible for generating circadian rhythmicity in mammals. Lesions of this bilateral structure abolish the normally occurring daily fluctuation of various functions, such as sleep-wakefulness, eating, locomotor activity and a number of hormonal rhythms²⁷.

One of the main targets of the SCN, receiving both vasoactive intestinal peptide VIP- and vasopressin (VP)-containing fibers, is the hypothalamic paraventricular dorsomedial nucleus (PVN/DMH)^{16,32,44,45}, which is regarded as an integrative site for neuroendocrine and autonomic functions^{20,35}. This structure also includes a prominent population of corticotropin-releasing factor (CRF)-containing cell bodies, which form a central component in the chain of neuroendocrine events leading to the ACTH-mediated release of adrenal glucocorticoids³⁵. Glucocorticoids are of critical importance for homeostasis, coordinating circadian events and restoring stress-induced disturbances^{9,21}. The prominent daily elevation of plasma corticosterone before the start of the active period is a well-known phenomenon^{6–8,19} which is abol-

ished after SCN lesions^{1,37,43}.

Of all demonstrated SCN transmitters VP and VIP are the two most prominent in a number of mammalian species^{3–5,22,33,38–41}, including man^{10,30}. Indeed, efferent SCN projections have been shown to contain either VP or VIP^{16,44}. To test the physiological significance of the projections from the SCN to the PVN/DMH area in controlling the daily plasma corticosterone peak we infused VP and VIP in the PVN/DMH complex. After lesioning the SCN, in order to completely remove its output, animals received a permanent indwelling jugular venous catheter and bilaterally implanted chronic cannulae above the PVN/DMH area to simultaneously allow frequent blood sampling and microinfusion in the brain¹⁵.

MATERIALS AND METHODS

Male Wistar rats (TNO Zeist, The Netherlands) were used in all studies. They were kept in a temperature-controlled environment (20–22°C) on a 12 h light/12 h dark schedule (07.00 h lights on). Animals were housed in individual cages (38 × 26 × 16 cm), with food and water available ad libitum.

A total of 120 animals of 180–200 g, anaesthetized with Hypnorm (Duphar, The Netherlands; 0.6 ml/kg, intramuscular), were mounted with their heads in a David Kopf stereotact with the toothbar set +5.0 mm, and sustained a bilateral lesion of the SCN

(coordinates: 1.4 mm rostral to bregma; 1.0 mm lateral to the midline; 8.4 mm below the brain surface). In the following three weeks effectiveness of the lesions was checked by measuring water intake during the last 8 h of the light period (09.00–17.00 h) and recording daily locomotor activity. Activity was recorded automatically by registering the number of interruptions of two crossed infrared beams, and could be displayed per any desired time period. Arrhythmic animals were subsequently provided with two chronic guide cannulae aimed at the PVN/DMH area (coordinates with flat skull: 1.8 mm caudal to bregma; 2.0 mm lateral to the midline; 7.0 mm below the brain surface), and a silicone catheter in the right atrium via jugular venectomy according to Steffens³¹. Experiments were started after one week recovery time, in which the animals were accustomed to the experimental procedures.

Experiment 1

At the start of an experiment the animal was removed from its home cage (between 08.00 and 08.30 h) and two 30 gauge stainless steel needles (o.d. 0.28 mm), connected to a Carnegie CMA/100 microinjection pump via two PP.10 polythene tubes (Portex Ltd., UK) filled with infusion medium, were inserted into the brain cannulae under light ether anaesthesia together with an adjustment of a PP.100 tube to the venous catheter. Thereafter the animal was transferred to an experimental cage (25 × 25 × 30 cm). Blood samples (0.2 ml) were taken within 1 min after taking the animal from its home cage, and 15 and 30 min after the ether stress. Afterwards the animal was allowed to adapt to the cage for 1 h. Ringer solution, VP (50 pg/min), VIP (200 pg/min), or VP-antagonist (50 pg/min) was infused into the PVN/DMH area for 15 min (40 nl/min). Blood samples (0.8 ml) were taken 10 and 0 min before, and 10, 20, 30 and 60 min after the start of the infusion. Each sample immediately being replaced by heparinized donor blood. The blood samples were collected in heparinized tubes placed in ice, centrifuged and the plasma stored at -20°C. Each animal was used only once weekly.

Experiment 2

During the second part of the experiments it was necessary to make a small modification to the experimental protocol in order to achieve basal pre-infusion levels of plasma corticosterone. Animals were now transferred to the experimental cage at the end of the day preceding the morning of the experiment and at the same time the infusion needles were inserted (so they stayed in overnight). Thereafter the procedure was similar to the previous protocol, i.e. animals were connected to the infusion pump via PP.10 polythene tubes filled with the infusion medium together with the adjustment to the venous catheter under light ether anaesthesia 2 h before the start of the infusion.

Experiment 3

In the final part of the experiments SCN-lesioned and intact animals were subjected to an arousing stimulus, i.e. the transfer to a novel cage. Animals were left overnight in an experimental cage and connected to the polyethylene blood sampling tube at 09.00 h. Blood samples (0.2 ml) were taken before ($t = -60, -45, -30$ and -15 min), and after ($t = 1, 5, 10, 15, 30, 45$ and 60 min) the transfer to another experimental cage at 11.00 h.

At the end of the experiments the animals were perfused with buffered 4% paraformaldehyde, the brains sectioned on a Vibratome, and the sections stained for VP, VIP or Nissl to analyse the position of the cannulas and the extent of the SCN lesions.

Plasma corticosterone was measured directly without prior extraction using commercially available [¹²⁵I]corticosterone radioimmunoassay kits (ICN Biomedical Division, Carson, CA, USA). The intra-assay (at 50% binding on the standard curve) and inter-assay coefficients of variation were 7.4 and 6.8%, respectively. The lower limit of sensitivity using this method is 2 ng/ml.

Fig. 1. Inhibitory effect of arginine-vasopressin (AVP) infusion in the PVN/DMH area on elevated plasma corticosterone levels. The insertion of the infusion needles and the light ether anesthesia to allow this procedure causes a rapid increase in plasma corticosterone levels (left panel of figure). 15-min infusions (shaded area) started 1 h later. Control infusions with Ringer's caused no changes in the corticosterone levels. Infusion of AVP in the SCN-lesioned animals, however, caused an immediate arrest of corticosterone release resulting in basal levels at 30 min after the start of the infusion, but were ineffective in SCN-intact animals. Intact/Ringer's, $n = 7$; Intact/AVP, $n = 8$; SCNx/Ringer's, $n = 8$; SCNx/AVP, $n = 9$.

RESULTS

Out of the total of 120 lesioned animals 41 proved to be completely arrhythmic during the course of this study and were used for PVN/DMH cannula placement.

Both intact and SCN-lesioned animals show basal plasma corticosterone levels when taken from their home cage (Fig. 1, arrow), although the values of the lesioned animals are significantly higher than that of intact controls. The insertion of the infusion needles and the light ether anesthesia to allow this procedure causes a rapid increase in the plasma corticosterone levels (left panel of Fig. 1). Control infusions with Ringer's, started 1 h after the ether anesthesia, caused no changes in the elevated corticosterone levels. Infusion of VP in the SCN-lesioned animals, however, caused an immediate arrest of corticosterone release resulting in basal levels at 30 min after the start of the infusion, but was ineffective in SCN-intact animals or in animals showing cannula placement outside the PVN/DMH area (data not shown). MANOVA and post-hoc analysis with the Student's t -test revealed that the VP-infused, SCN-lesioned rats differed significantly from all other groups at $t = 20$ and $t = 30$. Infusion of VIP did not affect the elevated corticosterone levels in either intact or SCN-lesioned animals, and were similar to the Ringer's infusion (data not shown).

For the second part of the experiment only animals with plasma corticosterone levels below 150 ng/ml at $t =$

Fig. 2. Stimulatory effect of infusion of the AVP antagonist in the PVN/DMH area on basal plasma corticosterone levels in intact animals. 15-min (shaded area) infusion of the AVP antagonist caused an immediate increase of plasma corticosterone levels in the intact animals, whereas in SCN_x animals the effects of the AVP antagonist and Ringer's on plasma corticosterone levels were similar. Intact/Ringer's, $n = 13$; Intact/AVP, $n = 10$; SCN_x/Ringer's, $n = 8$; SCN_x/AVP, $n = 8$.

0 were included in the analysis (Fig. 2). For the intact animals this meant 23 of the 28 infused animals, and for the SCN-lesioned animals, 16 out of 24. In the intact animals infusion of the VP antagonist (i.e. the V1 antagonist $d(\text{CH}_2)\text{Tyr}(\text{Me})\text{AVP}$, 50 pg/min) caused an immediate increase of plasma corticosterone levels, which remained elevated until at least 120 min after the start of the infusion. Ringer's infusion only caused an increment of corticosterone plasma levels at $t = 60$. In the SCN-lesioned animals infusion of both the VP antagonist and Ringer's caused an increase of plasma corticosterone levels, reaching peak levels at $t = 60$. MANOVA

Fig. 3. Patterns of plasma corticosterone levels in SCN-intact (○) and SCN-lesioned (●) rats 1 h before and 1 h after being placed in a new cage. Multivariate analysis of variance (MANOVA) showed significant effects of group, $F_{1,10} = 6.34$, $P < 0.05$, and time, $F_{10,100} = 2.59$, $P < 0.01$. Post-hoc analysis using the Student's t -test showed significant differences between groups at $t = 30$, 45 and 60. Post-hoc comparisons with paired t -tests showed that SCN-intact controls differed ($P < 0.01$) from the $t = -15$ value only at $t = 10$ and $t = 15$, whereas in the SCN-lesioned animals all plasma corticosterone values after the change of cages, except $t = 0$, differed from $t = -15$. Control, $n = 5$; SCN_x, $n = 7$.

followed by the Student's t -test revealed that in the intact animals corticosterone values differed at all time points during both conditions (i.e. infusion of VP antagonist or Ringer's), whereas in the SCN-lesioned animals both infusions evoked similar changes. Furthermore, corticosterone values of SCN-lesioned animals differed from that of the antagonist- or Ringer's-infused intact animals at $t = 20$, 30 and 120 min.

Under basal conditions SCN-intact animals showed normal daytime corticosterone values (i.e. < 50 ng/ml). The mean values of the SCN-lesioned animals, however, were about twice as high (Fig. 3.). The confrontation with the new cage induced a small increase in plasma corticosterone levels in the SCN-intact animals which declined after 15 min. On the other hand, the SCN-lesioned animals showed a more pronounced increase which had still not declined 1 h after the change to the new cage.

DISCUSSION

Previous studies have shown that at the level of the anterior pituitary, VP (derived from the PVN) facilitates the release of ACTH and thereby stimulates the release of corticosterone^{12,26}. However, some early studies also reported an inhibitory effect of VP on the release of either CRF^{24,42} or corticosterone²³. Since VP was applied intraventricularly in these studies the exact site or mechanism of action remained unknown. The present results clearly indicate that the inhibitory action of VP is localized in the PVN/DMH area. The localization of this

structure immediately adjacent to the third ventricle explains the effectiveness of the previous experiments employing i.c.v. application. Furthermore, the VP projection responsible for the inhibitory action of VP in the PVN/DMH area could be shown to originate from the SCN.

Lesioning of the SCN results in the loss of a large number of circadian rhythms (for review see 27), and the concomitant disappearance of VP- and VIP-containing fibers in a restricted number of areas, among which is the PVN/DMH area of the hypothalamus¹⁶. Re-instatement of part of this innervation, viz. by microinfusion of the peptidergic SCN transmitters in the PVN/DMH complex enabled us to investigate the importance of this projection in the regulation of one diurnal changing parameter, i.e. plasma corticosterone^{6-8,19}.

The substantial decrease of plasma corticosterone as seen after a 15 min infusion of VP (Fig. 1) indicates that VP in the PVN/DMH area is able to immediately shut off the release of corticosterone from the adrenal, since corticosterone levels decrease with the same half-life as observed after a bolus injection of corticosterone¹⁷. VP infusion was ineffective, however, in intact animals (Fig. 1), indicating that at the moment of testing, the VP receptors in the intact rat were already occupied. Question remains then, as to why stimulation of PVN VP receptors by exogenous VP decreases elevated corticosterone levels, whereas occupation of the same receptors by endogenous VP (as is the case in the intact animals of Fig. 1) does not. The difference might be explained by the fact that in intact animals these receptors are chronically occupied during the daytime, whereas in our SCN-lesioned animals VP is applied acutely to long-term, VP-deprived receptors. As has been shown for a number of receptor types VP receptors may also show changes in affinity after being deprived of their original input³⁴.

The data from Experiment 1 implicated to us an inhibitory role of VP-containing SCN efferents in the PVN/DMH complex on the release of corticosterone. Consequently a blockade of the VP-receptors in this hypothalamic area of intact animals with a VP antagonist, during the same day time period (i.e. 09.00–11.00 h), should remove the inhibitory tone from the SCN, and thereby increase plasma corticosterone levels. Furthermore, if VP is indeed derived from the SCN, such an infusion should be ineffective in SCN-lesioned animals. Therefore, in the second set of experiments we infused the VP V1 antagonist d(CH₂)Tyr(Me)AVP or Ringer's in intact and SCN-lesioned animals (Fig. 2.). In intact animals the arousal by the Ringer's infusion and blood withdrawal procedure only had a small effect on plasma corticosterone levels at $t = 60$. On the other hand, the combination of arousal and blocking of VP receptors

produced a robust and rapid increase in corticosterone levels, lasting at least 2 h, suggesting that an inhibitory input on corticosterone release had been removed. In contrast, in SCN-lesioned animals infusion of the VP antagonist and Ringer's resulted in plasma corticosterone increments which were very much alike, suggesting that in these animals endogenous VP was no longer inhibiting corticosterone release. At first sight the plasma corticosterone increments in the SCN-lesioned animals seem surprising. But, in fact, the Ringer's-infused SCN-intact animals were the only ones in which the inhibitory influence from the SCN was not blocked, and which also did not respond to the infusion procedure with a large increase of plasma corticosterone. The lack of complete overlap between the response of the antagonist infused SCN-intact animals and SCN-lesioned animals may be explained by the fact that in intact animals there is a sudden removal of inhibitory tone, while in SCN-lesioned rats the long-term lack of inhibitory input from the SCN may produce permanent changes in the target neurons of the PVN/DMH complex, e.g. at the level of the mRNA, as has been shown for other afferent inputs^{2,7,14,36}.

The fact that the infusion of VP is only effective in SCN-lesioned animals and the VP antagonist only in intact animals indicates that it is really SCN-derived VP which is responsible for the inhibition of the release of corticosterone, and not VP from the parvocellular neurons in the PVN. It is unclear, however, whether this is an effect directly on CRF neurons or rather on interneurons in the PVN/DMH area that reach the CRF neurons. Further anatomical and electrophysiological studies will be needed to investigate this problem.

The fact that in SCN-lesioned animals corticosterone levels are not chronically elevated up to extreme levels (Figs. 2 and 3) indicates that of course there are other systems inhibiting the release of corticosterone besides the VP efferents from the SCN. For example, corticosterone itself acts at the level of the pituitary to inhibit the synthesis and release of ACTH, as well as at the level of the hypothalamus to inhibit the synthesis and release of CRF⁷. In addition, inhibitory inputs to the hypothalamo-pituitary-adrenocortical system have been shown to originate in septum² and hippocampus^{13,28}. Furthermore, the inhibitory role of the SCN on corticosterone release is supported by the results of Experiment 3 showing that SCN-lesioned animals confronted with an arousing situation produced corticosterone excursions that are exaggerated and decline more slowly as compared to SCN-intact animals (Fig. 3). In fact, a result very much similar was obtained by Sapolsky et al.²⁸ using hippocampus-lesioned animals. In addition, contrary to intact animals, SCN-lesioned animals show frequent

excursions of basal plasma corticosterone levels during the light period^{1,37,43}. Therefore, it seems that together with the inhibitory systems described earlier, the SCN, too, acts to limit the magnitude and duration of corticosterone elevations. The major differences being that the SCN inhibition does not seem to be dependent on the feedback information of corticosterone levels, and probably acts merely during the daytime when elevated corticosterone levels may be even more harmful^{9,21}.

Of course, despite the fact that during the daytime the release of corticosterone is inhibited by the VP projection from the SCN to the PVN/DMH complex, still situations can be observed in which corticosterone levels are elevated during the daytime, as evidenced by the elevated values in the intact animals in Fig. 1, and the various stress experiments performed during the daytime^{11,18}. Therefore it can be concluded that the inhibitory influence from the SCN is not an absolute one, but can be encompassed by other systems to promote corticosterone release, such as e.g. an ascending projection from the brainstem^{7,36}.

The prominent VP rhythm as observed in the CSF²⁵, which probably originates from the SCN²⁹, entirely fits with the presently proposed inhibitory role for the SCN and VP on corticosterone release. Highest levels of CSF-VP (originating mainly from VP fibers lining the ventricular system) coincide with basal levels of corticosterone in the beginning of the light period^{6,8}. Moreover,

as VP levels in the CSF decline, plasma corticosterone slowly rises, reaching peak levels with the onset of the dark period when CSF levels of VP have their nadir. However, since the hypothalamo-pituitary-adrenal axis is not chronically activated in SCN-lesioned animals (as discussed above), this means that the circadian drive from the SCN cannot be explained solely by the presently described inhibiting VP projection. Although the results from Experiment 3 and other studies^{1,37,43} show that the SCN is important for keeping plasma corticosterone levels low during the daytime, it is clear that, in addition, there has to be yet another SCN mechanism able to excite the secretion of corticosterone in the evening.

In conclusion, the present data indicate that the VP projection from the SCN is yet another input to the PVN involved in the regulation of balanced corticosterone levels. The inhibitory effect of SCN-derived VP on the release of corticosterone suggests the need for future studies to examine the possibility that this projection forms part of the mechanism to induce the well-known circadian fluctuations in plasma corticosterone.

Acknowledgements. This study was supported in part by the Van Houten Fund of the NIBR. We thank Drs. D.F. Swaab, R. Balazs and F.J.H. Tilders for their helpful comments on earlier versions of this manuscript, and H. Stoffels and H. Overdijk for preparing the illustrations and developing the activity monitoring cages, respectively.

REFERENCES

- 1 Abe, K., Kroning, J., Greer, M.A. and Critchlow, V., Effects of destruction of the suprachiasmatic nuclei on the circadian rhythms in plasma corticosterone, body temperature, feeding and plasma thyrotropin, *Neuroendocrinology*, 29 (1979) 119–131.
- 2 Baldino, F., O'Kane, T.M., Fitzpatrick-McElligott, S. and Wolfson, B., Coordinate hormonal and synaptic regulation of vasopressin messenger RNA, *Science*, 241 (1988) 978–981.
- 3 Card, J.P. and Moore, R.Y., The suprachiasmatic nucleus of the golden hamster: immunohistochemical analysis of cell and fiber distribution, *Neuroscience*, 13 (1984) 415–431.
- 4 Card, J.P., Brecha, N., Karten, H.J. and Moore, R.Y., Immunocytochemical localization of vasoactive intestinal polypeptide-containing cells and processes in the suprachiasmatic nucleus of the rat: light and electron microscopic analysis, *J. Neurosci.*, 11 (1981) 1289–1303.
- 5 Card, J.P., Fitzpatrick-McElligott, S., Gozes, I. and Baldino, F., Localization of vasopressin-, vasoactive intestinal polypeptide-, peptide histidine isoleucine- and somatostatin mRNA in rat suprachiasmatic nucleus, *Cell Tissue Res.*, 252 (1988) 307–315.
- 6 Critchlow, V., Liebelt, R.A., Bar-Sela, M., Mountcastle, M. and Lipscomb, H.S., Sex difference in resting pituitary-adrenal function in the rat, *Am. J. Physiol.*, 205 (1963) 807–815.
- 7 Dallman, M.F., Akana, S.F., Cascio, C.S., Darlington, D.N., Jacobson, L. and Levin, N., Regulation of ACTH secretion: variation on a theme of B, *Rec. Prog. Brain Res.*, 43 (1987) 113–173.
- 8 De Boer, S.F. and van der Gugten, J., Daily variations in plasma noradrenaline, adrenaline and corticosterone concentrations in rats, *Physiol. Behav.*, 40 (1987) 323–328.
- 9 Devenport, L., Knehans, A., Sundstrom, A. and Thomas, T., Corticosterone's dual metabolic actions, *Life Sci.*, 45 (1989) 1389–1396.
- 10 Dierickx, K. and Vandesande, F., Immunocytochemical localization of the vasopressinergic and the oxytocinergic neurons in the human hypothalamus, *Cell Tissue Res.*, 184 (1977) 15–27.
- 11 Dunn, J., Scheving, L. and Millet, P., Circadian variation in stress-evoked increases in plasma corticosterone, *Am. J. Physiol.*, 222 (1972) 402–406.
- 12 Gillies, G.E., Linton, E.A. and Lowry, P.J., Corticotropin-releasing activity of the new CRF is potentiated several times by vasopressin, *Nature*, 299 (1982) 355–357.
- 13 Herman, J.P., Schäfer, M.K.H., Young, E.A., Thompson, R., Douglass, J., Akil, H. and Watson, S.J., Evidence for hippocampal regulation of neuroendocrine neurons of the hypothalamo-pituitary-adrenocortical axis, *J. Neurosci.*, 9 (1989) 3072–3082.
- 14 Herman, J.P., Wiegand, S.J. and Watson, S.J., Regulation of corticotropin-releasing hormone and arginine vasopressin messenger ribonucleic acid expression in the paraventricular nucleus: effects of selective hypothalamic deafferentations, *Endocrinology*, 127 (1990) 2408–2417.
- 15 Hermes, M.L.H.J., Buijs, R.M., van Heerikhuizen, J.J., van den Born, J. and van der Woude, T., Oxytocin neurotransmission in the A1-area of the brainstem induces hormonal vasopressin release in rats, *Eur. J. Neurosci.*, 1 (1989) 148–153.
- 16 Hoorneman, E.M.D. and Buijs, R.M., Vasopressin fiber pathways in the rat brain following suprachiasmatic nucleus lesioning, *Brain Res.*, 243 (1982) 235–241.

- 17 Kaneko, M. and Hiroshige, T., Fast, rate-sensitive corticosteroid negative-feedback during stress, *Am. J. Physiol.*, 234 (1978) R39-R45.
- 18 Kant, G.J., Mougey, E.H. and Meyerhoff, J.L., ACTH, prolactin, corticosterone and pituitary cyclic AMP responses to repeated stress, *Pharmacol. Biochem. Behav.*, 32 (1989) 557-561.
- 19 Krieger, D.T. and Hauser, H., Comparison of synchronization of circadian corticosteroid rhythms by photoperiod and food, *Proc. Natl. Acad. Sci. USA*, 75 (1978) 1577-1581.
- 20 Luiten, P.G.M., Ter Horst, G.J. and Steffens, A.B., The hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism, *Prog. Neurobiol.*, 28 (1987) 1-54.
- 21 Munck, A., Guyre, P.M. and Holbrook, N.J., Physiological functions of glucocorticoids in stress and their relation to pharmacological actions, *Endocrine Rev.*, 5 (1984) 25-44.
- 22 Okamura, H., Murakami, S., Uda, K., Sugano, T., Takahashi, Y., Yanaihara, C., Yanaihara, N. and Ibata, Y., Co-existence of vasoactive intestinal peptide (VIP)-, peptide histidine isoleucine amide (PHI)-, and gastrin releasing peptide (GRP)-like immunoreactivity in neurons of the rat suprachiasmatic nucleus, *Biomed. Res.*, 7 (1986) 295-299.
- 23 Pavel, S., Cristoveanu, A., Goldstein, R. and Calb, M., Inhibition of release of corticotropin releasing hormone in cats by extremely small amounts of vasotocin injected into the third ventricle of the brain: evidence for the involvement of 5-hydroxytryptamine-containing neurons, *Endocrinology*, 101 (1977) 672-678.
- 24 Plotsky, P.M., Bruhn, T.O. and Vale, W., Central modulation of immunoreactive corticotropin-releasing factor secretion by arginine vasopressin, *Endocrinology*, 115 (1984) 1639-1641.
- 25 Reppert, S.M., Schwartz, W.J. and Uhl, G.R., Arginine vasopressin: a novel peptide rhythm in cerebrospinal fluid, *Trends Neurosci.*, 10 (1987) 76-80.
- 26 Rivier, C. and Vale, W., Modulation of stress-induced ACTH release by corticotropin-releasing factor, catecholamines and vasopressin, *Nature*, 305 (1983) 325-327.
- 27 Rusak, B. and Zucker, I., Neural regulation of circadian rhythms, *Physiol. Rev.*, 59 (1979) 449-526.
- 28 Sapolsky, R.M., Krey, L.C. and McEwen, B.S., Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress response, *Proc. Natl. Acad. Sci. USA*, 81 (1984) 6174-6177.
- 29 Schwartz, W.J. and Reppert, S.M., Neural regulation of the circadian vasopressin rhythm in cerebrospinal fluid: a pre-eminent role for the suprachiasmatic nuclei, *J. Neurosci.*, 5 (1985) 2771-2778.
- 30 Sofroniew, M.V. and Weindl, A., Identification of parvocellular vasopressin and neurophysin neurons in the suprachiasmatic nucleus of a variety of mammals including primates, *J. Comp. Neurol.*, 193 (1980) 659-675.
- 31 Steffens, A.B., A method for frequent sampling blood and continuous infusion of fluids in the rat without disturbing the animal, *Physiol. Behav.*, 4 (1969) 833-836.
- 32 Stephan, F.K., Berkley, K.J. and Moss, R.L., Efferent connections of the rat suprachiasmatic nucleus, *Neuroscience*, 6 (1981) 2625-2641.
- 33 Swaab, D.F., Pool, C.W. and Nijveldt, F., Immunofluorescence of vasopressin and oxytocin in the rat hypothalamo-neurohypophyseal system, *J. Neural. Transm.*, 36 (1975) 195-215.
- 34 Swank, M.W. and Dorsa, D.M., Chronic treatment with vasopressin analogues alters affinity of vasopressin receptors in the septum and amygdala of the rat brain, *Brain Res.*, 544 (1991) 342-344.
- 35 Swanson, L.W., The Hypothalamus. In A. Björklund, T. Hökfelt and L.W. Swanson (Eds.), *Handbook of Chemical Neuroanatomy, Vol. 5., Integrated Systems of the CNS, Part 1*, Elsevier, Amsterdam, 1989, pp. 1-124.
- 36 Swanson, L.W. and Simmons, D.M., Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: a hybridization histochemical study in the rat, *J. Comp. Neurol.*, 285 (1989) 413-435.
- 37 Szafarczyk, A., Ixart, G., Malaval, F., Nougues-Soule, J. and Assenmacher, I., Effects of lesions of the suprachiasmatic nuclei and of *p*-chlorophenylalanine on the circadian rhythms of adrenocorticotrophic hormone and corticosterone in the plasma, and on locomotor activity of rats, *J. Endocrinol.*, 83 (1979) 1-16.
- 38 Tillet, Y., Caldani, M. and Tramu, G., Immunohistochemical characterization of the sheep suprachiasmatic nucleus, *J. Chem. Neuroanat.*, 2 (1989) 215-226.
- 39 Ueda, S., Kawata, M. and Sano, Y., Identification of serotonin- and vasopressin immunoreactivities in the suprachiasmatic nucleus of four mammalian species, *Cell Tissue Res.*, 234 (1983) 237-248.
- 40 van den Pol, A.N. and Tsujimoto, K.L., Neurotransmitter of the hypothalamic suprachiasmatic nucleus: immunocytochemical analysis of 25 neuronal antigens, *Neuroscience*, 15 (1985) 1049-1086.
- 41 Vandesande, F., Dierickx, K. and De Mey, J., Identification of the vasopressin-neurophysin producing neurons of the rat suprachiasmatic nuclei, *Cell Tissue Res.*, 56 (1975) 377-380.
- 42 van Dijk, A.M.A., Lodewijks, M.M.J.M., van Ree, J.M. and van Wimersma Greidanus, T.B., Inhibitory and stimulatory action of vasopressin on the secretion of corticotrophin in rats: structure-activity study, *Life Sci.*, 29 (1981) 1107-1116.
- 43 Watanabe, K. and Hiroshige, T., Phase relation between episodic fluctuations of spontaneous locomotor activity and plasma corticosterone in rats with suprachiasmatic nuclei lesions, *Neuroendocrinology*, 33 (1981) 52-59.
- 44 Watts, A.G. and Swanson, L.W., Efferent projections of the suprachiasmatic nucleus. II. Studies using retrograde transport of fluorescent dyes and simultaneous peptide immunohistochemistry in the rat, *J. Comp. Neurol.*, 258 (1987) 230-252.
- 45 Watts, A.G., Swanson, L.W. and Sanchez-Watts, G., Efferent projections of the suprachiasmatic nucleus. I. Studies using anterograde transport of *Phaseolus vulgaris* leuco-agglutinin in the rat, *J. Comp. Neurol.*, 258 (1987) 204-229.