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GABA Receptors in the Region of the Dorsomedial Hypothalamus of Rats Are Implicated in the Control of Melatonin and Corticosterone Release

Andries Kalsbeek
Willem-Jan Drijfhout[b]
Ben H.C. Westerink[b]
Joop J. van Heerikhuize[a]
Tjitske P. van der Woude[a]
Jan van der Vliet[a]
Ruud M. Buijs[a]

[a] Netherlands Institute for Brain Research, Amsterdam, and
[b] University Centre for Pharmacy, Groningen, The Netherlands

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Abstract
Recently, anatomical evidence was presented that the mammalian circadian clock located in the suprachiasmatic nuclei (SCN) may utilize GABA to transmit diurnal information to the dorsomedial hypothalamus (DMH). The present study provides further physiological evidence for the involvement of this GABAergic projection in the regulation of diurnal rhythms. Infusion of the GABA agonist muscimol in the region of the DMH completely blocked the daily increase of plasma melatonin during darkness and reduced sympathetic output in the pineal gland resulting in lower pineal melatonin production, as measured with transpineal microdialysis. Further experiments in SCN-lesioned animals indicated that the origin of this inhibitory input to the DMH is indeed the SCN. The results of this study imply that the SCN can influence the sympathetic outflow of the hypothalamus through its GABA-containing projection. Furthermore, the present results probably explain the previously reported strong inhibitory effect of benzodiazepines on plasma melatonin in both animals and humans.

Introduction

The main oscillator for the mammalian brain, the biological clock, is located in the suprachiasmatic nuclei of the hypothalamus (SCN) [1]. Via its efferent projections, the output signal of the SCN is translated into a wealth of behavioral, physiological and endocrine rhythms. Usually, the phase of the circadian oscillator is estimated on the basis of the rhythm of one of these output signals, such as locomotor activity, body temperature or corticosterone concentrations. Yet another important marker of the circadian phase, not readily disturbed by masking conditions such as stress, activity of feeding, is the daily rhythm of the pineal hormone melatonin. Despite its important functions in circadian and reproductive physiology [2] and its recent use as a marker of SCN function [3, 4] the neural pathways involved in the regulation of the circadian rhythm in pineal activity are only partly known.

The daily melatonin rhythm is entrained by light to the prevailing photoperiod. In addition, light is also able to acutely suppress melatonin production at night. Light acts on the SCN through the retina and the retinohypothalamic tract (RHT). Information from the SCN reaches the sympathetic system via a not well established pathway in which the dorsal hypothalamus takes a central position [5–8]. Release of pineal melatonin at night is finally stim-

KARGER
E-Mail karger@karger.ch
Fax + 41 61 306 12 34

0028–3835/96/0631–0069$10.00/0

A. Kalsbeek
Netherlands Institute for Brain Research
Meibergdreef 33
NL–1105 AZ Amsterdam (The Netherlands)

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ulated by sympathetic nerve fibers originating in the superior cervical ganglion [9]. Using the gonadal responses to changing photoperiods as an indicator, knife cut studies have implicated both projections from the SCN towards the dorsal hypothalamus and descending hypothalamic projections in the control of pineal activity [10–14]. A number of (peptidergic) transmitter candidates have been identified in SCN neurons and their efferent projections [15–17]. Some of these have been implicated in the regulation of melatonin secretion [18–20], but as yet it is not known which one of these transmits the inhibitory effect of light onto the melatonin rhythm generating system in the dorsal hypothalamus.

Recently, a τ-aminobutyric acid (GABA)-containing projection from the SCN to the paraventricular and dorsomedial hypothalamus was described [21]. In addition, the GABA-containing innervation of the dorsomedial and posterior hypothalamus has been implicated in the control of sympathetic outflow [22–26]. Accordingly, the present study was undertaken to determine whether the GABAergic projection from the SCN might be involved in depressing the activity of neurons in the hypothalamus which are associated with the control of the circadian melatonin rhythm. To assess the role of GABA, a GABA-A receptor antagonist (Bicuculline; BIC) and agonist (Muscimol; MUS) were administered to the dorsal hypothalamic area (DMH) by retrodialysis (i.e. the delivery of substances locally within a tissue via a microdialysis probe). In order to assess whether the GABAergic projection of the SCN was involved infusions were also performed in SCN-lesioned animals. Melatonin release was monitored either indirectly by taking blood samples or directly by transpineal microdialysis [27].

Material and Methods

Male Wistar rats (TNO Zeist, The Netherlands) were used in all experiments. They were kept in a temperature-controlled environment (20–22 °C) on a 12-hour light/12-hour dark schedule [lights on at 07.00 h (SCN-lesions), 22.00 h (experiment 1) or 18.00 h (experiment 2)]. Before the start of the actual experiments animals were allowed to acclimatize to the lighting schedule for several weeks with 4–6 animals per cage. During experiments animals were housed in individual cages (38 × 26 × 16 cm), with food and water available ad libitum.

For experiment 1, a total of 20 animals of 180–200 g, anesthetized with Hypnorm (Duphar, The Netherlands; 0.6 ml/kg i.m.), were mounted with their heads in a David Kopf stereotact with the toothbar set at +5.0 mm, and sustained a bilateral lesion of the SCN (coordinates: 1.4 mm rostral to bregma; 1.0 lateral to the midline; 8.2 mm below the brain surface). In the following 3 weeks, the effectiveness of the lesions was checked by measuring water intake during the middle 8 h of the light period (09.00–17.00 h). Only animals showing a water intake of >30% during the middle part of the light period were used for further experiments. SCN-lesioned (n = 11) and intact control animals (n = 8) were provided with a microdialysis probe and a silicone catheter in the right atrium when their body weight was >300 g. The microdialysis probe was stereotaxically implanted with its U-shaped end positioned just lateral to the DMH (ccordinates with flat skull: 2.8 mm caudal to bregma; 1.6 mm lateral to the midline; 8.0 mm below the brain surface). The dialysis probe consisted of a dialysis fiber (molecular weight cut-off 6,000 Da, 3 mm total length), glued into the ends of two parallel 25-gauge stainless steel tubings. The loop of the probe was positioned in the rostrocaudal direction along the DMH. The jugular venectomy was performed according to the method of Steffens [28]. Probe and atrial outlet were secured in place with dental cement to two stainless steel screws inserted in the skull. Experiments were started after a recovery time of 1 week. In order to accustom the rats to the conditions of the experiments they were permanently connected to the dialysis and blood-sampling system of catheters protected by a metal sheath and kept away from from the animals by means of a counterbalanced beam. This allowed normal movements, sleep, feeding, body weight recovery, and growth.

For experiment 2, 10 animals were provided with a microdialysis probe aimed at the DMH as described above and a transpineal probe according to Drijfhout et al. [27]. Animals were allowed to recover overnight and connected to the perfusion pump and injection valve of the HPLC apparatus via a dual-channel swivel (Carnegie) the next morning.

Experiment 1

On the morning of the experiment at 08.00 h (i.e. CT10), animals were connected to the syringe pumps via a piece of PE20 tubings. The probe was perfused continuously with Ringer at a flow rate of 200 μl/h. Ringer solution was exchanged for Ringer, BIC (100 μ*M*) or MUS (10 μ*M*) at CT13.40, perfused for 3 h and changed back to Ringer at CT16.40 for an additional 3 h. Lagtime from syringe to the brain was 20 min. Ringer, BIC or MUS were applied in a random order. Blood samples (0.8 ml) were taken just before the onset of darkness at CT11.5 and at CT15, CT16, CT17 and CT19. Each sample was immediately replaced with heparinized donor blood. The blood samples were collected in heparinized tubes placed in ice, and centrifuged. The plasma was stored at –20 °C.

At the end of the experiment the animals were perfused with buffered 4% paraformaldehyde, the brains sectioned on a vibratome, and the sections stained for vasopressin (VP), vasoactive intestinal polypeptide (VIP) or Nissl to analyze the extent of the SCN lesions and the position of the dialysis probe.

Plasma corticosterone was measured directly without prior extraction using commercially available [^{125}I]corticosterone radioimmunoassay kits (ICN Biomedical Division, Carson, Calif., USA). The intraassay (at 50% binding on the standard curve) and interassay coefficients of variation were 7.4 and 6.7%, respectively. The lower limit of sensitivity using this method is 2 ng/ml.

Plasma melatonin concentrations were measured in duplicate by radioimmunoassay (RIA) using [^{125}I]melatonin (Amersham, Bucks., UK; specific activity 2,000 Ci/mmol) and a rabbit antiserum (AB/R/03, Stockgrand Ltd., Guilford, UK) at a final dilution of 1:160,000. Stock melatonin (Sigma Chemicals) was stored at a concentration of 1 mg/ml. Melatonin was extracted from plasma samples (250 μl) in 5 ml dichloromethane and dried by vacuum evapora-

tion. Next, samples were reconstituted in 150 µl assay buffer and two 50-µl aliquots were taken for assay. Standards were diluted in assay buffer to give a range of dilutions from 0.5 to 200 pg/ml. The minimum detection limit levels for the assay were between 5 and 10 pg/ml plasma.

Experiment 2

The transpineal probe was perfused with Ringer continuously at a flow rate of 200 µl/h. Samples were collected every 20 min and injected on-line on a HPLC system by means of an automated injector. Melatonin content of the dialysate was measured by fluorometric detection (Ex = 280 nm, EM = 345 nm). Infusions via the dialysis probe in the DMH were performed as described above, but now MUS infusions lasted maximally 1 h.

The response time patterns of plasma melatonin and corticosterone were evaluated with analysis of variance (MANOVA). Differences between SCN-lesioned and intact controls were analyzed with a two-way MANOVA (group vs. time) followed by Student's t tests on independent measures to find out at which time points the two groups differed. Within experimental groups differences between treatments (i.e. Ringer, MUS or BIC infusion) were analyzed by a two-way MANOVA with both treatment and time as repeated measures within subject factors followed, if F values were appropriate, by a Student's t test on paired samples. To find within treatment groups which time points differed from 'time 0' values, a one-way MANOVA was performed followed by a Student's t test (paired). It was decided that a p value smaller than 0.05 was significant. However, when used repeatedly to compare various time points in one experiment the Student's t test has a real probability treshold much higher than its nominal threshold (i.e. 0.05), therefore the Student's t test was applied using the Bonferroni correction.

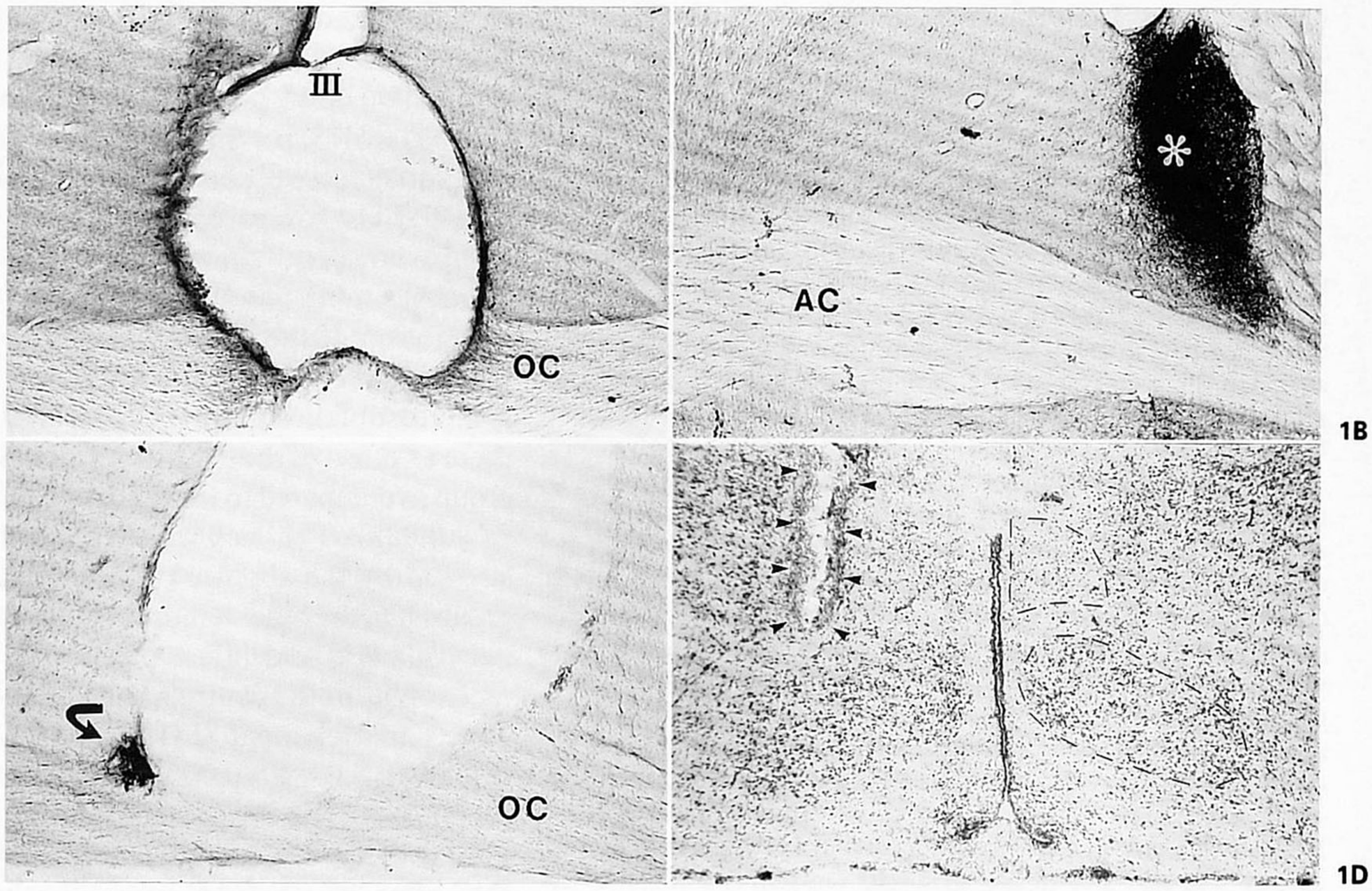
Results

In weeks 2 and 3 after having sustained a bilateral lesion of the SCN, 11 of the 20 animals consumed >30% of their drinking water during the middle 8 h of the light period (mean = 36.0 ± 1.6%, rest mean = 15.5 ± 1.8%). These 11 lesioned and another 8 intact animals were provided with an intracerebral microdialysis probe and jugular catheter. One SCN-lesioned and 2 control animals had to be removed later on due to problems with either their dialysis probe or jugular catheter. One SCN-lesioned animal was removed from the experimental group due to an incomplete lesion (fig. 1). Therefore, the results of 9 SCN-lesioned and 6 intact control animals were used for final analysis.

During Ringer infusion plasma melatonin levels at CT11.5 were all below 10 pg/ml in control animals. After onset of darkness melatonin concentrations increased from CT15 onwards, reaching peak values of about 30 pg/ml at CT17 and CT19. All SCN-lesioned animals showed fairly constant values during the whole sampling period. Multivariate analysis of the no-drug condition in both groups showed no significant group difference ($F(1,13) = 0.45$, $p = 0.515$), but did reveal a significant effect of time ($F(4,52) = 6.59$, $p < 0.001$) and an interaction effect ($F(4,52) = 5.75$, $p = 0.001$). Student's t tests showed a significant increase of plasma melatonin in the control group at CT17 as compared to CT11.5. The SCN-lesioned group, however, showed no such diurnal variations (fig. 2A).

Infusion of the GABA-antagonist BIC from CT14 to CT17 in the control group did not affect the normal nightly increase of melatonin ($F(1,5) = 0.78$, $p = 0.418$), nor was there an effect of the 3 h BIC infusion in the SCN-lesioned group as compared to the no-drug situation ($F(1,8) = 0.01$, $p = 0.933$). The lack of effect of the BIC infusion is stressed by the absence of an interaction effect in both groups. On the other hand, a 3-hour infusion of the GABA agonist MUS did affect the normal release pattern of melatonin. In the control group MANOVA showed clear effects of treatment ($F(1,5) = 7.71$, $p = 0.039$), time ($F(4,20) = 5.07$, $p = 0.006$) and an interaction of both factors ($F(4,20) = 4,53$, $p = 0.009$). After the MUS infusion, plasma melatonin levels were significantly lower at CT17 than after the Ringer infusion. Furthermore, contrary to the no-drug and BIC infusion, at no time did plasma melatonin levels differ from that at CT11.5, explaining the strong 'time × treatment' interaction. In the SCN-lesioned group there was only a clear effect of treatment ($F(1,8) = 12.52$, $p = 0.008$) during infusion of MUS. Paired t tests showed significantly lower melatonin values at CT17 as compared to the no-drug situation (fig. 3A).

Plasma corticosterone values in the control group showed a decreasing trend from CT11.5 onwards. However, this 'time' effect just escaped significance ($p = 0.078$). No such trend was observed in the SCN-lesioned group ('time' $p = 0.410$). Due to the large variation in the SCN-lesioned group no significant differences could be detected between the no-drug corticosterone levels of the control group and the SCN-lesioned group (fig. 2B). Changes in plasma corticosterone induced by the infusions of MUS and BIC were completely the opposite of the changes observed in plasma melatonin. In neither group did MUS infusion affect corticosterone secretion as compared to the no-drug situation ('control' treatment: $F(1,5) = 0.72$, $p = 0.436$; time: $F(4,20) = 2.32$, $p = 0.092$; interaction: $F(4,20) = 1.84$, $p = 0.161$; 'SCN-lesioned' treatment: $F(1,8) = 0.02$, $p = 0.888$; time: $F(4,32) = 2.04$, $p = 0.112$; interaction: $F(4,32) = 0.25$, $p = 0.905$). In both groups, however, the 3-hour BIC infusion caused significant increases in corticosterone secretion ('control' treatment: $F(1,5) = 17.89$, $p = 0.008$; time: $F(4,20) = 8.39$,



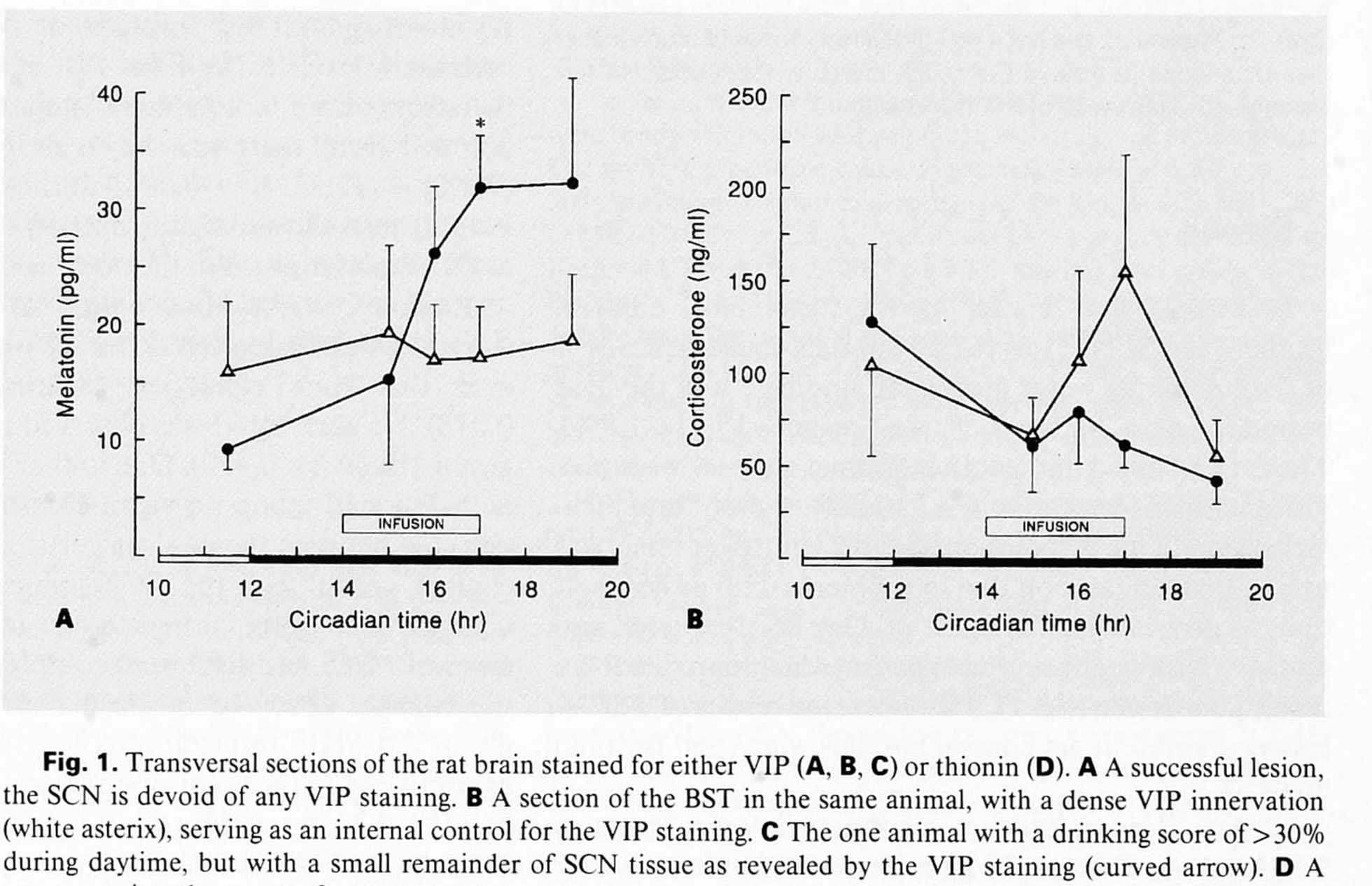


Fig. 1. Transversal sections of the rat brain stained for either VIP (**A**, **B**, **C**) or thionin (**D**). **A** A successful lesion, the SCN is devoid of any VIP staining. **B** A section of the BST in the same animal, with a dense VIP innervation (white asterix), serving as an internal control for the VIP staining. **C** The one animal with a drinking score of >30% during daytime, but with a small remainder of SCN tissue as revealed by the VIP staining (curved arrow). **D** A representative placement of a DMH probe (outlined by arrowheads). At the contralateral side both the VMH and DMH are outlined to facilitate orientation. OC = Optic chiasm; AC = anterior commisure; III = third ventricle.

Fig. 2. Plasma melatonin (**A**) and corticosterone (**B**) levels in intact control (●) and SCN-lesioned (△) animals during the first half of the dark period. The box marked 'infusion' indicates the three-hour period of Ringer infusion. * $p < 0.05$ vs. CT11.5.

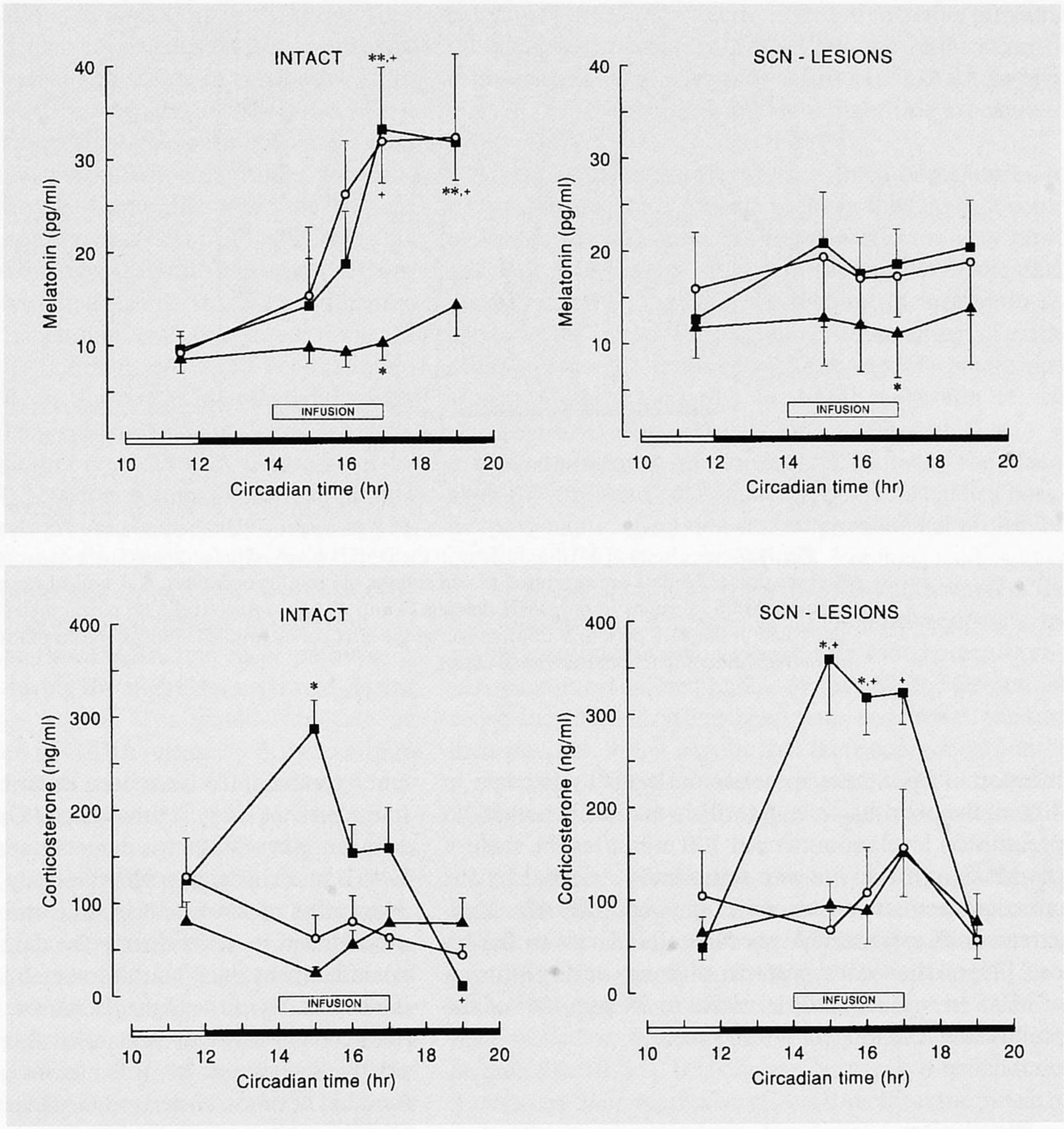


Fig. 3. Effect of MUS and BIC infusions in intact control and SCN-lesioned animals on plasma melatonin (**A**) and corticosterone (**B**) levels. The box marked 'infusion' indicates the three-hour period during which either Ringer (○), MUS (▲) or BIC (■) was infused. * $p < 0.05$ vs. Ringer; ** $p < 0.05$ BIC vs. MUS; $^{+}$ $p < 0.05$ vs. CT11.5.

$p < 0.001$; interaction: $F(4,20) = 8.38$, $p < 0.001$; 'SCN-lesioned' treatment: $F(1,8) = 10.48$, $p = 0.012$; time: $F(4,32) = 16.13$, $p < 0.001$; interaction: $F(4,32) = 8.56$, $p < 0.001$). Especially in the SCN-lesioned group corticosterone increases were pronounced during infusion of BIC in the DMH, but abated quickly when the infusate was changed to Ringer again (fig. 3B).

Experiment 2

On-line measurement of pineal melatonin release by transpineal dialysis was performed between CT18 and CT22. Basal melatonin values during the preinfusion period were comparable to the ones reported previously, i.e. 19.2 fmol/min. Infusion of MUS in the DMH caused an immediate and rapid decrease of melatonin levels detected by the transpineal dialysis (fig. 4A). A 40-min

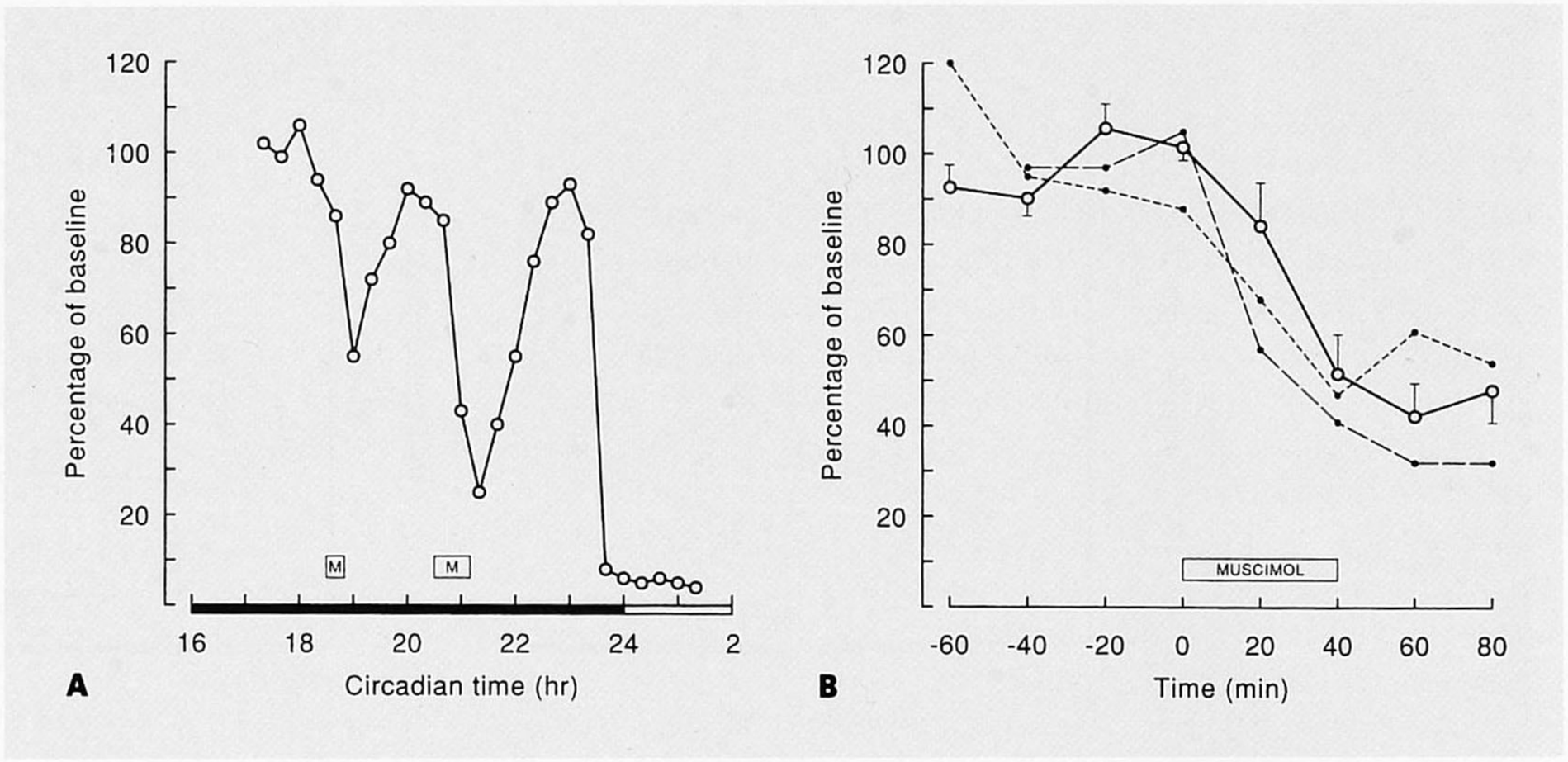


Fig. 4. Acute effects of MUS infusions in the DMH on pineal melatonin content as measured with a transpineal dialysis probe. All data are expressed as percentages of preinfusion values. **A** A typical example of the effect of two separate MUS infusions in the DMH (lasting 20 and 40 min, respectively) on pineal melatonin content during the latter half of the dark period. **B** Comparison of the effect of 40 min MUS infusions on pineal melatonin (n = 6) and noradrenaline content (two individual cases).

infusion of MUS caused melatonin levels to decrease to 40% of the preinfusion levels within 60 min. Recovery to preinfusion levels commenced 100 min after the start of the MUS infusion, but was sometimes obscured by the circadian decrease at the end of the night (fig. 4B). Measurement of extracellular noradrenaline levels in the pineal [29] on the second postoperative way during infusion of MUS (n = 2) showed decreases to 30 and 40% of the preinfusion level.

Discussion

GABA is an important inhibitory neurotransmitter in the hypothalamus. It has been estimated that in some nuclei about half of the total synaptic input may be accounted for by GABA-containing terminals [30]. The source(s) of these extensive GABAergic inputs to the hypothalamus, however, are largely unknown. In addition to the abundant intranuclear or locally derived GABAergic innervation [31], recent studies have provided examples of more distant origins for the GABA-containing input to the hypothalamus. In addition to the recently described GABA-containing projection from the SCN to PVN and DMH [21], GABAergic projections from the BNST and basal forebrain to, respectively, PVN and lateral hypothalamus have been described as well [31–33]. In the present study, a pronounced GABAergic inhibition of melatonin release was demonstrated at the level of the DMH. In accordance with previously published data [34–36] plasma melatonin levels in control animals showed a pronounced increase during the dark period, apparently not affected by the 3-hour intracerebral Ringer infusion at the beginning of the night. However, infusion of MUS in the DMH completely prevented this nightly increase of plasma melatonin. In order to investigate the putative function of the SCN-derived GABAergic projection to the DMH in this inhibition of melatonin release, the experiments were continued with SCN-lesioned animals. Ablation of the SCN completely eliminated the normal nightly increase in plasma melatonin. Instead, a constant release pattern was found slightly above basal daytime values (fig. 2A). Initial studies indicated that SCN ablation resulted in complete abolition of the pineal NAT rhythm and constant low levels throughout the 24-hour day [37, 38]. Subsequent studies indicated consistently low levels of pineal and plasma melatonin as a consequence of SCN lesions as well [6, 7]. However, in all of these studies relatively few time points were sampled and often pooled data were used. Using continuous CSF sampling in SCN-lesioned monkeys, Reppert et al. [39] did find abolition of the circadian rhythm but, contrary to the rodent data, also

considerable fluctuations in melatonin levels throughout the day/night cycle. Only very recently Tassonneaud et al. [40] showed similar results in sheep. In sheep with complete lesions of the SCN, the characteristic nocturnal melatonin peak was replaced by a continuous melatonin secretion, clearly above normal daytime values, throughout the day/night cycle. Combined with the present results, these data suggest that ablation of the SCN results in a disinhibition of the hypothalamic melatonin rhythm generating system, eliminating the rhythmic influences but allowing basal melatonin synthesis to continue. In accordance with this, Lehman et al. [8] demonstrated that, whereas lesions in the dorsal hypothalamus completely eliminated the nightly increase of the pineal melatonin content, ablation of the SCN only caused a partial decrease. Since in SCN-lesioned animals elevated plasma melatonin concentrations are significantly suppressed by the infusion of MUS into the DMH, we propose that the GABA-containing projection from the SCN to the DMH transmits an inhibitory signal on melatonin secretion.

It is feasible that this GABAergic SCN pathway is largely suppressed during the night but activated during the (subjective) day period. This would implicate an endogenous variation in GABA release by SCN terminals comparable to VP and somatostatin [41]. Indeed, circadian fluctuations in hypothalamic and SCN GABA content or turnover have been described [42–44], a rhythm which persisted in constant darkness [44]. On the other hand, it is also possible that GABA release by SCN terminals is only stimulated by the perception of light. Hence, the definitive proof for the physiological significance of GABA release in the DMH depends on abolition of either the circadian decline in plasma melatonin values during (subjective) day or prevention of the suppressive effect of light exposure on plasma melatonin through the blockade of GABA receptors in the DMH. The absence of BIC-induced changes in melatonin release in the present series of experiments indicates that there is no endogenous secretion of GABA during the dark period in intact animals. On the other hand, it is also possible that melatonin synthesis and release are already at their maximum and cannot be further increased by blockade of a possible remaining inhibitory input. In this regard, it has been shown with transpineal microdialysis that local infusion of the β-agonist isoprenaline via the infusion medium stimulates the release of melatonin when administered during daytime [27] but not during the dark period [unpubl. observations]. The lack of a BIC-induced increase of melatonin secretion in the SCN-lesioned animals is in line with the removal of the GABAergic input to the DMH by ablation of the SCN projection. We are currently pursuing this line of experiments, i.e. to test the implication of endogenous GABA release by infusion of GABA antagonists in the DMH during day- and nighttime exposure to light.

Using a dual-probe approach, with co-implantation of a transpineal and an infusion probe in the DMH, it could be shown that the onset of the MUS effect is very rapid and that MUS, when infused in the middle of the dark period, acutely suppresses the amount of melatonin released by the pineal. Furthermore, measurement of extracellular pineal NA levels showed decreases that were very similar to those observed in pineal melatonin release (fig. 4B). Therefore, the inhibitory effect of GABA is mediated via the sympathetic innervation of the pineal gland. The present data, however, do not exclude a possible presynaptic effect of a central pinealopetal projection on the sympathetic nerve endings. Previous studies have shown a tonic inhibitory effect of GABA at the level of the hypothalamus on a number of other components of sympathetic outflow as well, i.e. heart rate, blood pressure and plasma noradrenaline [22, 23, 25, 26]. Since SCN lesions cause increased daytime heart rate and blood pressure measures [45, 46], it may be that these components of the sympathetic system are under the inhibitory control of an SCN-derived GABAergic input to the hypothalamus as well.

The present results also provide an explanation for some previous findings with respect to the depressive effects of benzodiazepines on circulating melatonin concentrations. A number of studies in the human have shown a suppressive effect of benzodiazepines taken at bedtime on subsequent plasma melatonin levels [47–49, but see also 50, 51]. It is unclear, however, if benzodiazepines exert their depressive effects directly at the pineal or at a retinal or central level. Despite the extensive literature on benzodiazepine effects on circadian rhythms [52], the in vivo effects of benzodiazepines on pineal activity have to date been investigated only rarely in animals, probably because the phase-shifting action of triazolam was not prevented by pinealectomy [53]. In 1979, Zatz and Brownstein [54], however, described a pronounced depressive effect of i.p. administered diazepam on pineal NAT levels. The present study confirms the conclusion of the authors at that time that '... these (GABAergic) drugs act in the hypothalamus distal to the suprachiasmatic nucleus to interfere with the transmission of the stimulus which causes increased adrenergic stimulation of the rat pineal gland ...'. More precisely, our results show that the dorsomedial hypothalamus contains

a population of GABA-sensitive neurons responsible for the sympathetic drive to melatonin synthesis. Thus, centrally acting benzodiazepines may interfere with the normal circadian rhythms of melatonin release.

Apart from a GABAergic inhibition of hypothalamic sympathetic outflow, pronounced effects on the secretion of pituitary hormones have also been described [55–58]. Therefore, next to melatonin also infusion effects on the corticosterone rhythm were studied. Despite previous results of ourselves and others [58, 60] the present study revealed no clearcut circadian corticosterone rhythm. The decreasing trend of plasma corticosterone levels observed after the circadian peak at the onset of darkness just missed significance ($p = 0.08$), probably due to the arousal caused by the subsequent infusion experiment. In fact, the ready disturbance of plasma corticosterone levels by experimental procedures is one of the reasons why more and more plasma melatonin levels are used as a marker of SCN activity instead of other (hormonal) outputs [3, 4]. The primary goal of the present study was to study the effect of GABA on the melatonin rhythm and thus blood samples were mainly taken during the dark period. However, a blood sample taken during the trough of the circadian corticosterone rhythm (i.e. CT0 to CT6) certainly would have revealed the circadian rhythm in control animals more clearly. In addition, variation was considerably enhanced in SCN-lesioned animals. Due to the abolition of the inhibitory SCN influence plasma concentrations may vary between basal daytime and increased nighttime values [61]. Thus, even if a clear circadian rhythm is present in control animals a significant effect of the SCN lesion may be hard to detect due to its inevitable effect of enhanced variability. In case of the melatonin data MANOVA indicated a clear effect of 'time' in the control group and an interaction of 'group versus time', but still Student's t tests detected no significant differences due to the large variation in the data of SCN-lesioned animals.

Intracerebroventricular administration of GABA antagonists has revealed a tonic inhibitory GABAergic tone on the HPA axis [56, 62–64]. The present results confirm and extend the previous data by showing that a GABAergic projection responsible for the tonic inhibition of the HPA axis is directed at the dorsomedial hypothalamus. These data infer that apart from the previously proposed inhibitory projection from the DMH to the parvocellular PVN [21], and excitatory projection exists as well. Indeed, anatomical and physiological studies have provided evidence for both inhibitory and excitatory transmitters in the DMH projection to the parvocellular PVN. Whereas infusion of galanin in the PVN has been shown to inhibit CRF release, infusion of neurotension stimulates the release of CRF and ACTH [65–71]. The prolonged elevation of plasma corticosterone levels in the SCN-lesioned animals as compared to controls after infusion of the GABA antagonist, once again stresses the strong inhibitory influence of the SCN on the CRF secretory system reported previously [59–61]. The effectiveness of BIC-induced elevations in plasma corticosterone in both SCN-lesioned and intact controls indicates that the GABAergic projection responsible for inhibition of corticosterone release is not derived from the SCN. In accordance with this, infusion of MUS in SCN-lesioned animals did not induce a decrease of the elevated corticosterone levels (contrary to its effect on plasma melatonin levels). Thus, SCN regulation of the circadian rhythmicity in corticosterone and melatonin release are clearly separated phenomena. Whereas the VP-containing fibers in the SCN projection to the DMH inhibit the release of corticosterone, the GABA-containing part of this projection inhibits the release of melatonin but does not seem to affect the release of corticosterone. The present data do not reveal the origin of the GABA-containing projection to the DMH responsible for the inhibitory effect on the HPA axis. Several projections inhibitory to the HPA axis have been proposed to originate from limbic areas such as hippocampus, septum and BNST [72]. Both the BNST and amygdala projections are known to contain GABA [31, 32].

As revealed by our previous studies, the VP-containing projection from the SCN to the DMH plays an important role in the inhibitory effect on corticosterone release [59, 61]. Similarly, evidence is accumulating that an SCN-derived VIP-containing projection is involved in the control of the preovulatory LH surge [73–75]. At present, however, it is not clear in which SCN target area the VIP effect on LH release is exerted. In addition to the peptidergic control of the circadian rhythmicity in hormone release, the present study provides evidence for a GABAergic control of the circadian rhythm in melatonin release, again at the level of the DMH.

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