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# Melatonin analogues as agonists and antagonists in the circadian system and other brain areas

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**Abstract**

We studied the effects of drugs related to melatonin on neuronal firing activity in the suprachiasmatic nucleus, intergeniculate leaflet and other brain areas in urethane-anesthetized Syrian hamsters. We tested melatonin and two naphthalenic derivatives of melatonin, a putative agonist (S20098: *N*-[2-(7-methoxy-1-naphthyl)ethyl]acetamide), and a putative antagonist (S20928: *N*-[2-(1-naphthyl)ethyl]cyclobutyl carboxamide). Both melatonin and S20098 given intraperitoneally (i.p.) were able to suppress firing rates of cells in a similar dose-dependent manner, but the effects of S20098 were longer lasting. Iontophoresis of melatonin dose dependently depressed spontaneous and light-evoked activity of cells in the suprachiasmatic nucleus and intergeniculate leaflet, while iontophoresis of S20098 was relatively ineffective, probably because it is a poorly charged compound. S20928 (2.0–10 mg/kg, i.p.) alone decreased firing rates of light-sensitive cells by 25–50% for 5–30 min in the suprachiasmatic nucleus and intergeniculate leaflet; however, low doses (< 2.0 mg/kg) of S20928 partially blocked the effects of melatonin agonists on most cells. The non-selective serotonin antagonist metergoline did not block the effects of either melatonin agonist. Both melatonin agonists and antagonists were less effective when applied to cells in the hippocampus and dorsal lateral geniculate nucleus. These results indicate that S20098 is an agonist acting probably on melatonin receptors in the Syrian hamster brain. S20928 may have mixed agonist/antagonist properties, but at low doses appears to function as an antagonist at melatonin receptors in the suprachiasmatic nucleus and intergeniculate leaflet.

*Keywords:* Melatonin; Suprachiasmatic nucleus; Intergeniculate leaflet; Electrophysiology; Light; Circadian rhythm; Entrainment

## 1. Introduction

The suprachiasmatic nucleus of the hypothalamus functions as the dominant pacemaker for behavioral and physiological circadian rhythms in mammals (for review, see Rusak and Zucker, 1979; Moore, 1983; Meijer and Rietveld, 1989). Photic information required for entrainment to lighting cycles reaches the suprachiasmatic nucleus through a direct projection from the retina, the retinohypothalamic tract, and via an indirect pathway, the geniculohypothalamic tract, originating in the retinorecipient intergeniculate leaflet of the lateral geniculate nuclei (Harrington and Rusak, 1986; Pickard et al., 1987; Zhang and Rusak, 1989).

Many cells in the suprachiasmatic nucleus and intergeniculate leaflet respond to illumination of the whole retina with increases in firing rates, while a few show decreases (Meijer et al., 1986, 1992; Harrington and Rusak, 1989; Ying et al., 1993; Ying and Rusak, 1994). These cells are presumed to be involved in mediating responses of the circadian system to light.

The pineal hormone melatonin has been shown to play a role in the regulation of circadian rhythms in rats (Cassone, 1992; Cassone et al., 1986; Redman et al., 1983; McArthur et al., 1991) and Djungarian hamsters, apparently by acting on the suprachiasmatic nucleus, although possibly on other targets as well. Mela-

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tonin has also been shown to affect circadian rhythm phase-shifting and entrainment in humans (Branchy et al., 1982; Armstrong et al., 1993; Lewy et al., 1992); melatonin or its analogues may have potential as therapeutic agents for a variety of sleep and rhythm-related disorders (Tobler et al., 1994).

Previous studies have demonstrated that pressure ejection or iontophoresis of melatonin can alter firing rates of suprachiasmatic nucleus cells in a Syrian hamster brain slice preparation (Mason and Rusak, 1990; Yu et al., 1993; Rusak and Yu, 1993) and can suppress photic responses of cells in the intergeniculate leaflet (Ying and Rusak 1993). The conclusion that melatonin acts on suprachiasmatic nucleus cells is reinforced by the finding that the suprachiasmatic nucleus of many species contains high-affinity binding sites for melatonin (Duncan et al., 1988, 1989; Reppert et al., 1988; Weaver et al., 1991; for review, see Stankov and Reiter 1990; Morgan et al., 1994).

Studies of 2-[$^{125}$I]iodomelatonin binding have indicated that there are at least two different binding sites, one with picomolar affinity and the other with nanomolar affinity for melatonin, as demonstrated in chick brain and retina (Ying and Niles, 1991; Ying et al., 1992; for a review, see Krause and Dubocovich, 1991), and in the Syrian hamster brain (Dubocovich, 1988; Pickering and Niles, 1990; Maywood et al., 1995). Signal transduction studies have revealed that the high affinity sites of melatonin receptors are negatively coupled to activity of adenylate cyclase via a G protein (Carlson et al., 1989; Ying et al. 1992), while the nanomolar affinity sites are linked to stimulation of phosphoinositide breakdown via a G protein in chick brains (Popova and Dubocovich, 1995). However, in the hippocampus and cortex of some primates (Stankov et al., 1993) and ovine hippocampus (Barrett et al., 1994), the receptor is not linked to a G protein. Little is known, however, about the functions of these subtypes in circadian rhythm regulation, since selective agonists and antagonists for them are not yet available (for a review, see Morgan et al., 1994).

Pharmacological analysis of melatonin receptors in in vivo preparations is still at an early stage. Several drugs, such as luzindole, *N*-acetylserotonin (Dubocovich, 1988), and ML23 (Zisapel and Laudon, 1987), have been suggested to function as melatonin receptor antagonists, but these functional claims have not been substantiated, and the affinity of these drugs for melatonin receptors is very low, as demonstrated by 2-[$^{125}$I]iodomelatonin binding assays (for a review, see Morgan et al., 1994).

A series of naphthalenic analogues of melatonin have been synthesized recently and reported to have very high affinities for 2-[$^{125}$I]iodomelatonin binding sites (Yous et al., 1992; Bonnefond et al., 1993; Dubocovich et al., 1993). Previous results have shown that one of these compounds, *N*-[2-(7-methoxy-1-naphthyl)-ethyl] acetamide (S20098), can mimic the effects of melatonin in phase-shifting circadian rhythms of activity (Redman and Guardiola-Lemaître, 1993; Van Reeth et al., 1994). Its affinity has been studied for a broad range of receptors other than melatonin receptors ($n = 44$), and the $IC_{50}$'s are typically $> 10^{-5}$ M, except for two serotonin receptors (5-$HT_{1A}$: $4 \times 10^{-6}$ M; and 5-$HT_{2C}$: $6 \times 10^{-7}$ M) (Yous et al., 1992; unpublished observations). The other compound, *N*-[2-(1-naphthyl)ethyl]cyclobutyl carboxamide (S20928), may function as an antagonist. There is evidence that S20928 can block the inhibitory action of melatonin on forskolin-stimulated adenylate cyclase activity in sheep pars tuberalis cells, reverse the pigment aggregation induced by melatonin in *Xenopus* melanophores, and antagonize short-day induced (melatonin-mediated) weight gain in the garden dormouse (Delagrange et al., 1994). This compound is also selective for the melatonin receptor since its affinity for other receptors ($n > 30$) is very low ($IC_{50}$'s $> 10^{-5}$ M; unpublished observations).

These drugs are of interest because of their high affinity for melatonin receptors, their potential to help differentiate types of melatonin receptors, and the possibility that one may function as an antagonist. In the present study, we used electrophysiological recordings to investigate the effects of melatonin, the putative melatonin receptor agonist, S20098 and the putative antagonist, S20928, on firing rates of cells in the suprachiasmatic nucleus and intergeniculate leaflet, as well as on cells in the hippocampus and dorsal lateral geniculate nucleus in Syrian hamsters. A focus of interest was the effects of these drugs on the responses to photic stimulation of cells in the intergeniculate leaflet and suprachiasmatic nucleus, which are hypothesized to play a role in circadian rhythm entrainment.

## 2. Materials and methods

### 2.1. Animals

Male Syrian hamsters (Charles River, St. Constant, Québec) weighing 100–145 g were kept in a photoperiod with 14 h of light daily beginning at 05:00 for at least 2 weeks before being used.

### 2.2. Surgical procedure

Hamsters were anesthetized with 25% urethane (2 g/kg, i.p.), and given subcutaneous injections of Robinul (3-hydroxy-1,1-dimethylpyrrolidinium bromide α-cyclopentylmandelate, A.H. Robins; 0.2%, 0.1–0.2 ml/animal) to reduce congestion in the respiratory tract during anesthesia. Additional doses of the drugs

were given as required. Hamsters were mounted in a stereotaxic apparatus and body temperature was monitored and maintained at 37°C with a thermostatically controlled heating pad throughout the experiment. A hole was drilled in the skull overlying the suprachiasmatic nucleus or intergeniculate leaflet region, with the aid of a magnifier. Special care was taken to avoid bleeding caused by damage to the superior sagittal sinus. The eyelids on the side contralateral to the recording site were retracted with sutures and the eye was covered with mineral oil to prevent dehydration after the pupils were dilated with a topical application of 1% atropine sulfate. Electrodes were aimed at the suprachiasmatic nucleus using stereotaxic coordinates (0.2–0.6 mm anterior to bregma, 0.2–0.35 mm lateral to the midline, and 7.6–7.8 mm ventral to the cortical surface), with the upper incisor bar 2 mm below the interaural line. Electrodes aimed at the hippocampus, dorsal lateral geniculate nucleus and intergeniculate leaflet were inserted at 1.4–1.6 mm posterior to bregma, 3.3–3.8 mm lateral to the midline, and 2.0–4.5 mm ventral to the cortical surface. The hamster was maintained in a darkened room except during light pulses or while repositioning the electrode.

### 2.3. Extracellular recordings and drug applications

Five-barrel glass micropipettes were prepared as described previously (Ying et al., 1993), with the recording barrel filled with fast green (Sigma) at a subsaturated concentration in 2 M NaCl. One barrel was filled with 2 M NaCl for automatic current balancing. Each of the remaining three barrels contained one of the following compounds: melatonin hydrochloride (Sigma), metergoline (a gift of Dr. Chiara de Paolis, Farmitalia, Milan, Italy), and S20098 (Servier, Paris, France). Melatonin, S20098 and metergoline were dissolved in 100% DMSO (dimethyl sulfoxide, Sigma Chemicals, St. Louis, MO) and then further diluted with 0.9% NaCl solution or distilled water so that the final vehicle concentration was 2.5–20% (v/v). For i.p. injections, melatonin, S20098 and S20928 were dissolved in 100% DMSO and diluted with saline to a final concentration of 5–20% DMSO. All drug solutions were freshly prepared. Drug concentrations and pH were 5 mM and 4.0–5.0 for iontophoretic studies, and drug doses were 0.5–10.0 mg/kg for systemic treatments. Vehicle solutions containing DMSO at similar concentrations were used for control treatments.

Single-unit extracellular recordings were made from 15:00–20:00 h, overlapping both projected light and dark phases of the daily illumination cycle in the colony room. Contralateral whole-retinal illumination was conveyed to the eye from a tungsten-halogen lamp using fiber optics and a computer-controlled shutter, and photically responsive neurons were identified and recorded during sustained light presentations (typically 1–2 min), as described previously for studies of neurons in the intergeniculate leaflet and suprachiasmatic nucleus (Ying et al., 1993; Ying and Rusak, 1994).

Cells were defined as photically responsive or light-sensitive if their firing rates were consistently increased or suppressed by $> 30\%$ by sustained, whole-retinal illumination. Some cells classified as 'non-photic' by this criterion, especially in the dorsal lateral geniculate nucleus, might be sensitive to other patterns of illumination in restricted receptive fields, but this feature was not assessed.

Data acquisition, light exposures and iontophoretic applications of drugs were controlled by a computer program (Pulsecount) written by A.J. Hurshman. Data analysis and histological identification of recording sites were described previously in detail (Ying et al., 1993). Briefly, agonist effects were defined as repeatable changes relative to predrug firing rates of $> 20\%$ at some dose of a drug. In order to compare effects of melatonin and its analog, agonist potencies were also expressed as $ED_{50}$'s, the doses required to produce half maximal effects, as described previously (Lum and Piercey, 1988). The impact of the antagonist was expressed as the proportion of the effect of an agonist that was reversed by co-application of the antagonist; i.e., the difference between the agonist effects in the absence and presence of the antagonist, divided by the agonist effect alone, and expressed as a percentage.

## 3. Results

Iontophoresis of 20% dimethyl sulfoxide (vehicle) had only slight effects on firing activity in 60 cells

Table 1
Effects of melatonin and S20098 on firing rates of light-sensitive cells in hamster suprachiasmatic nucleus and intergeniculate leaflet

| | Effects on spontaneous activity | | Effects on photic responses | |
|---|---|---|---|---|
| | Melatonin | S20098 | Melatonin | S20098 |
| *Suprachiasmatic nucleus* | | | | |
| Decrease | 28 (70%) | 15 (39%) | 30 (75%) | 17 (44%) |
| Increase | 6 (15%) | 6 (15%) | 8 (20%) | 5 (13%) |
| No Effect | 6 (15%) | 18 (46%) | 2 (5%) | 17 (44%) |
| Total | 40 | 39 | 40 | 39 |
| *Intergeniculate leaflet* | | | | |
| Decrease | 30 (67%) | 12 (31%) | 35 (78%) | 12 (31%) |
| Increase | 6 (13%) | 5 (13%) | 8 (18%) | 3 (8%) |
| No Effect | 9 (20%) | 22 (56%) | 2 (4%) | 24 (61%) |
| Total | 45 | 39 | 45 | 39 |

Drugs (5.0 mM) were iontophoresed onto cells at currents of 60–80 nA for 3 min during darkness and light presentation. A change of $> 20\%$ from pre-drug firing rate was considered a drug effect.

Table 2
Comparison of S20098 effects on spontaneous firing rates of non-photically responsive cells in the suprachiasmatic nucleus, hippocampus and dorsal lateral geniculate nucleus

| | Suprachiasmatic nucleus | Hippocampus | Dorsal lateral geniculate nucleus |
|---|---|---|---|
| Decrease | 26 (65%) | 18 (40%) | 18 (42.8%) |
| Increase | 5 (12.5%) | 4 (8.9%) | 4 (9.5%) |
| No Effect | 9 (22.5%) | 23 (51.1%) | 20 (47.6%) |
| Total | 40 (100%) | 45 (100%) | 42 (100%) |

The melatonin receptor agonist, S20098, was injected in darkness (3.0 mg/kg, i.p.). A change of > 20% from pre-drug firing rate was considered a drug effect.

tested, probably because it is a poorly charged compound. Systemic injections of the vehicle, however, often caused changes in firing rates; about 30% of cells showed > 20% change in firing rates in response to vehicle treatments and were not included in the analysis of the data. Of the remaining cells, histological reconstruction of recording sites revealed that 75 photically responsive cells were located in the suprachiasmatic nucleus area (most of which were in the ventrolateral subdivision of the suprachiasmatic nucleus), and 83 were in the region of the intergeniculate leaflet, including the intergeniculate leaflet itself and the adjacent ventral lateral geniculate nucleus.

### *3.1. Effects of melatonin agonists on firing rates of photically responsive cells in the suprachiasmatic nucleus and intergeniculate leaflet*

The firing rates of most cells in both suprachiasmatic nucleus and intergeniculate leaflet were suppressed by iontophoresed melatonin; this occurred during both the 1–2 min light pulses and the intervening dark periods (Table 1; Fig. 4B). The degree of suppression was found to depend on the iontophoretic current over the range of 0–90 nA using 3 min ejection durations. Firing rates of a small subset of cells were increased by melatonin applications (Table 1). There was no apparent change in the shape or amplitude of spikes during drug ejections.

The melatonin agonist S20098 was less effective than melatonin in suppressing firing rates when applied iontophoretically (Table 1). Since S20098 is a very poorly charged compound which might not be appropriate for iontophoretic studies, we also applied both melatonin and S20098 by means of i.p. injections in order to compare their effects. The two compounds exhibited similar inhibitory effects on firing rates at doses of 0.5–5.0 mg/kg. The suppression was found to

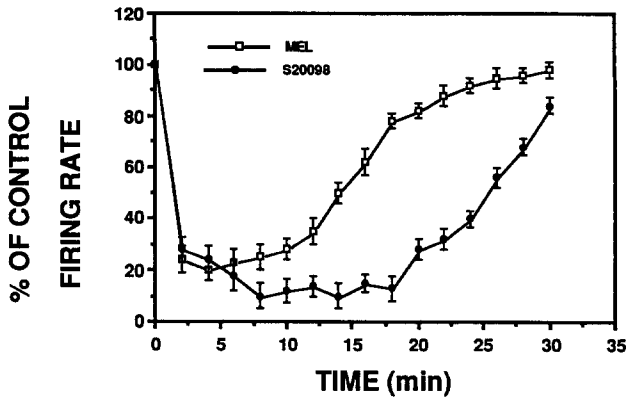


Fig. 2. Comparison of recovery times of light-sensitive suprachiasmatic nucleus cells after administration of melatonin (MEL) and S20098. S20098 was injected at a dose of 5 mg/kg in 0.25 ml vehicle one min before firing rates were recorded for 30 min. Each point represents the mean ± S.E.M., $n = 10$.

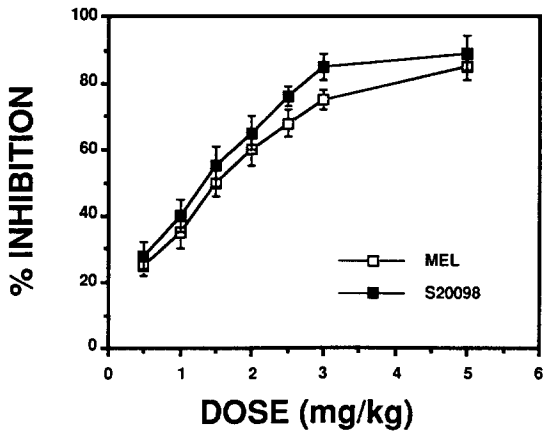


Fig. 1. Comparison of the effects of melatonin (MEL) and the agonist S20098 on spontaneous activity of light-sensitive suprachiasmatic nucleus cells in hamsters. Each drug was injected i.p. at doses of 0.5–5 mg/kg B.W. in 0.25 ml vehicle. A change of > 20% from pre-drug firing rates was considered a drug effect. Each point represents the mean ± S.E.M., $n = 3$–10.

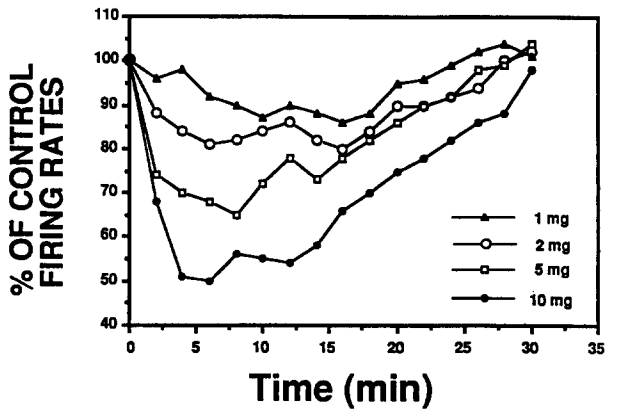


Fig. 3. Dose-related suppression of firing rates of cells in the suprachiasmatic nucleus and intergeniculate leaflet by the putative melatonin antagonist S20928 injected i.p. The drug was injected ~ 1 min before firing rates were recorded for 30 min. Each curve represents mean data from three to five cells, with error bars omitted for clarity.

be dose-dependent, as shown in the dose-response curves (Fig. 1). The $ED_{50}$, calculated from the dose-response curves, was 1.15 mg/kg for melatonin and 0.91 mg/kg for S20098. Latencies for these drugs to produce suppression (> 20%) of firing rates after i.p. injections were very similar (means ± S.E.M.: 85 ± 3 s for melatonin and 88 ± 4 s for S20098). After a single dose of 5.0 mg/kg, the suppressant effect of S20098 lasted longer than that of melatonin (Fig. 2). The time needed to attain 50% recovery of the baseline firing rate after drug administration was 26.5 min for S20098 and 13.1 min for melatonin. The sensitivity of photically responsive cells to melatonin and S20098 was similar in the suprachiasmatic nucleus and intergeniculate leaflet.

### 3.2. Effects of S20098 on spontaneous activity of non-photic cells in the suprachiasmatic nucleus, hippocampus and dorsal lateral geniculate nucleus

Most cells recorded in these brain areas were responsive to the agonist, S20098 (1.0–5.0 mg/kg, i.p.), with latencies and durations of drug effects similar to those observed in light-sensitive cells. However, a

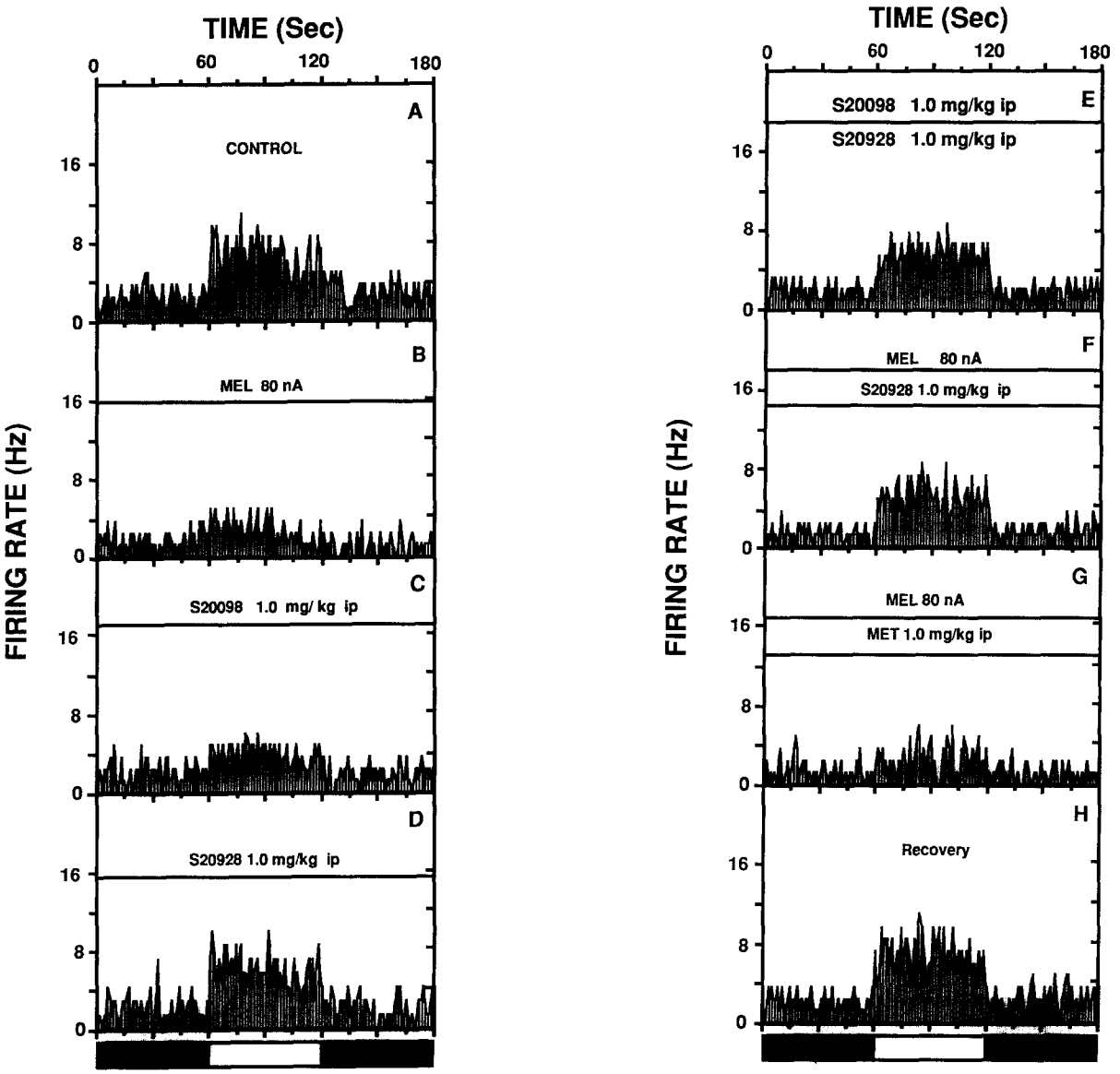


Fig. 4. Firing-rate histogram illustrating spontaneous activity and responses of a suprachiasmatic nucleus cell to light. (A) Control (no drug) response to light presentation. (B) Effects of iontophoresis of melatonin (5 mM). (C) Effects of i.p. S20098. (D) Effects of i.p. S20928. (E) Antagonism of the effects of S20098 injections on firing rate by S20928. (F) Antagonism of the effects of melatonin iontophoresis on firing rate by S20928. (G) Failure of the serotonin antagonist metergoline (MET) to antagonize the effects of melatonin. (H) Recovery of pre-drug photic responses. The white bar at the bottom of this and following figures indicates the timing of the light pulse.

Table 3
Effects of intraperitoneal injections of S20928 alone on firing rates of light-sensitive cells in the hamster suprachiasmatic nucleus and intergeniculate leaflet

| Dose (mg/kg) | Increase | Decrease | No effect | Total |
|---|---|---|---|---|
| 0.5 | 1 (4%) | 3 (11%) | 22 (85%) | 26 |
| 1.0 | 4 (9%) | 8 (17%) | 34 (74%) | 46 |
| 2.0 | 10 (19%) | 18 (34%) | 25 (47%) | 53 |
| 3.0 | 5 (14%) | 22 (59%) | 10 (27%) | 37 |
| 5.0 | 3 (7%) | 31 (70%) | 10 (23%) | 44 |
| 10.0 | 1 (4%) | 22 (92%) | 1 (4%) | 24 |

A change of > 20% from pre-drug firing rate was considered a drug effect. There were no significant differences ($t$-test, $P > 0.05$, $n = 30$) between the drug effects on suprachiasmatic nucleus and intergeniculate leaflet cells, nor during darkness and light exposure, so the data shown are pooled for both structures and both lighting conditions.

higher proportion of cells in the suprachiasmatic nucleus than in other brain areas were responsive to S20098 (Table 2).

### 3.3. Effects of the melatonin antagonist, S20928, on firing rates of photic suprachiasmatic nucleus and intergeniculate leaflet cells and non-photic cells

In our preliminary experiments, we attempted to apply S20928 by means of iontophoresis (0–100 nA, 3 min), and found that very few cells in the suprachiasmatic nucleus or other areas were responsive to the drug (data not shown). This drug appears to be even less readily applied by iontophoresis than S20098. Therefore, we studied its effects primarily using i.p. injections. Administration of S20928 alone, at doses of 0.5–2.0 mg/kg in 0.25 ml, had no or slight effect on firing rates of photically responsive cells tested in the suprachiasmatic nucleus and intergeniculate leaflet (Fig. 3). However, at doses from 2–10 mg/kg, apparent agonist effects were observed in most cells tested (Table 3, Fig. 3). The latencies and duration of effect were 1–2 min and 2–20 min, respectively, depending on the drug dose given. In order to test for whether S20928 had antagonist properties, we, therefore, applied the drug at doses no greater than 2 mg/kg when in combination with an agonist.

Table 4
S20928 antagonism of effects of melatonin and S20098 on firing rates of light-sensitive cells in the hamster suprachiasmatic nucleus and intergeniculate leaflet

| | Antagonism [a] | Partial antagonism [b] | No effect [c] | Total |
|---|---|---|---|---|
| *Suprachiasmatic nucleus* | | | | |
| Melatonin | 12 (44%) | 9 (33%) | 6 (22%) | 27 |
| S20098 | 16 (33%) | 12 (25%) | 20 (42%) | 48 |
| Total | 28 (37%) | 21 (28%) | 26 (35%) | 75 |
| *Intergeniculate leaflet* | | | | |
| Melatonin | 15 (36%) | 17 (41%) | 10 (24%) | 42 |
| S20098 | 13 (33%) | 13 (33%) | 14 (35%) | 40 |
| Total | 28 (34%) | 30 (37%) | 24 (29%) | 82 |

The melatonin receptor antagonist, S20928 alone (1.0–2.0 mg/kg B.W., i.p.) was administrated first to test its effects on firing rates, and then injected 1 min before an agonist was given by iontophoresis (60–80 nA, 3 min) or by i.p. injection (2.0–3.0 mg/kg) to assess its antagonist actions. Cells whose firing rates were affected by the antagonist alone by > 20% were not included.
[a] Agonist actions were reduced by more than 50%. [b] Agonist actions were reduced by 21–50%. [c] Agonist actions were unaffected ( < 20%) or even potentiated by the antagonist.

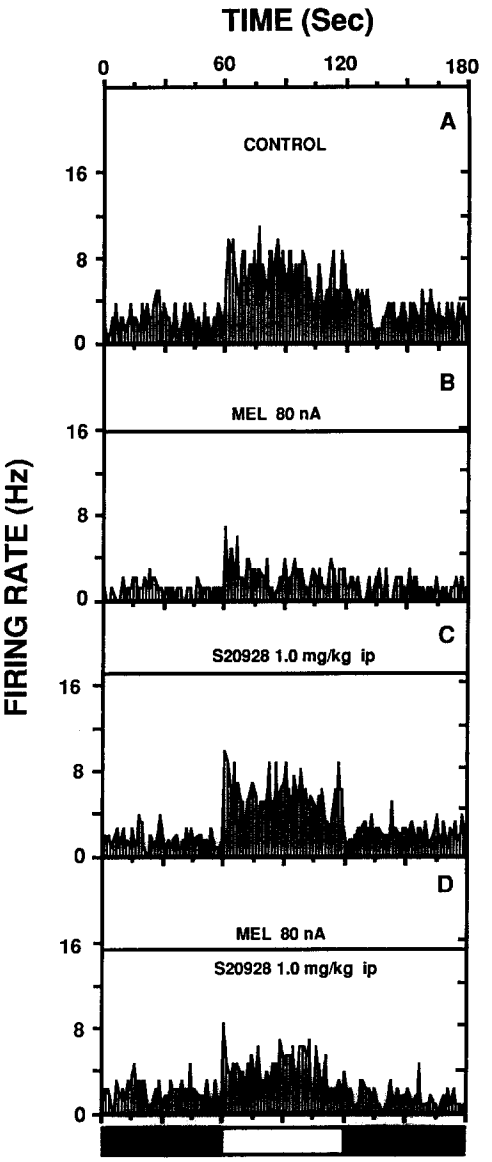


Fig. 5. Effects of S20928 on responses of a photically activated suprachiasmatic nucleus cell to melatonin (MEL). (A) Control response to light presentation. (B) Suppression of firing activity by melatonin. (C) Slight suppression of firing activity by S20928 alone. (D) Partial antagonism by S20928 of the suppressive effects of melatonin. See Fig. 4.

Effects of both melatonin (Fig. 4B) and S20098 (Fig. 4C) could be antagonized either potently (Fig. 4E,F) or partially (Fig. 5D) by S20928 at doses of 0.5–2.0 mg/kg. In a few cells tested, these low doses of S20928 either did not affect or even somewhat potentiated the actions of the agonists (Table 4). To assess the possibility that the observed effects of S20928 were non-specific or related to the vehicle, we tested whether a serotonin receptor antagonist (metergoline) or the 10–20% DMSO vehicle which was used to dissolve S20928 would mimic the effects of S20928. Although metergoline (1–2 mg/kg, i.p.) slightly increased firing rates of light-sensitive suprachiasmatic nucleus cells, as observed previously (Ying and Rusak, 1994), neither metergoline nor the vehicle alone was able to mimic the action of S20928 in antagonizing the effects of melatonin agonists on the same cells (Fig. 4G).

Furthermore, S20928 was found to be less potent as an antagonist for non-photic cells. It only partially blocked (< 45% reversal) or even potentiated responses to melatonin and S20098 of cells in the hippocampus ($n = 20$) and dorsal lateral geniculate nucleus ($n = 20$), as well as non-photic suprachiasmatic nucleus cells ($n = 20$) (Fig. 6).

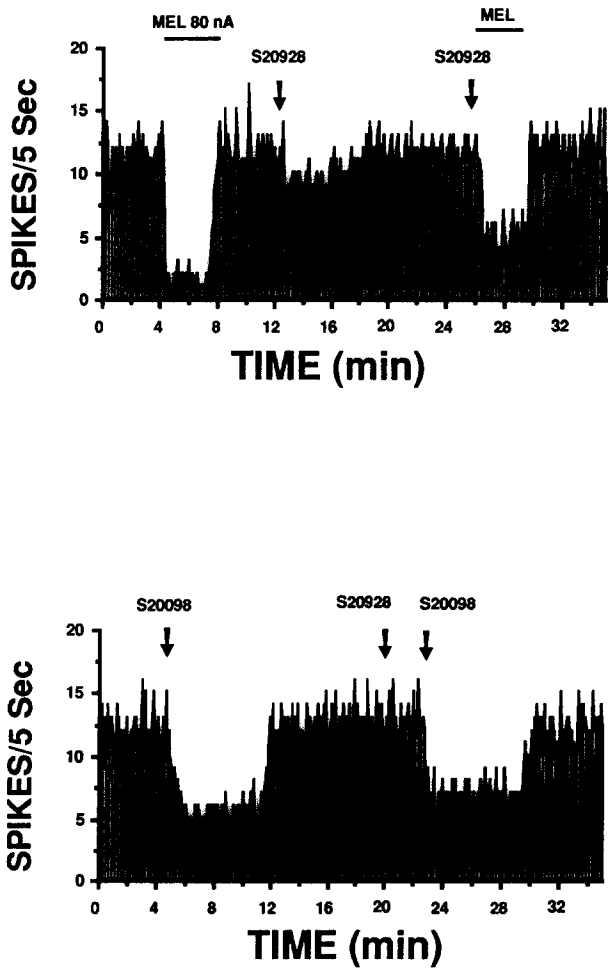


Fig. 6. Partial antagonism by the melatonin antagonist S20928 of responses to iontophoresis of melatonin (MEL) and the melatonin agonist S20098 in a single hippocampal cell. S20928 was injected i.p. at a dose of 1.0 mg/kg and 2 min later melatonin (5 mM, 80 nA) was iontophoresed or S20098 was injected i.p. (1.0 mg/kg). The interval between the two recordings was 15 min.

## 4. Discussion

The present results show that S20098, when given i.p., mimicked effects of melatonin on the spontaneous and light-evoked firing rates of photically responsive suprachiasmatic nucleus and intergeniculate leaflet cells in hamsters. Its effects were roughly equivalent in amplitude to those of melatonin, but with a somewhat prolonged duration of action. This increased duration of action probably reflects different kinetics in its association with its receptor(s), or a somewhat different metabolic pathway. Recent behavioral studies have found that S20098 can phase shift circadian activity rhythms in rats, mice, and Syrian hamsters as melatonin does, but even more effectively (Redman et al., 1995; Van Reeth et al., 1994). Therefore, our data strongly suggest that S20098 acts as a melatonin receptor agonist in neural components of the circadian system of Syrian hamsters. The relative ineffectiveness of S20098 during attempted iontophoretic applications appears to be related to its poor solubility and inability to become electrically charged. As a result, only very small amounts of the drug were likely ejected by bulk flow along with charged ions or by electro-osmosis in the vehicle solution (Hicks, 1984).

The fact that many of our findings were based on intraperitoneal injections raises questions about the site of action of these drugs, such as whether effects on photic responses might be mediated via the retina rather than directly in the suprachiasmatic nucleus. The assumption that S20098 and S20928 act directly on suprachiasmatic nucleus cells is reinforced by evidence from a smaller number of suprachiasmatic nucleus cells in this study that direct iontophoretic applications of either S20098 or melatonin had effects similar to those of systemic injections, and by evidence that S20098 (1 mM), applied by micropressure ejection to suprachiasmatic nucleus cells in an in vitro slice preparation, also suppresses cell firing (G. Scott and B. Rusak, unpublished observations).

In addition, we demonstrated that S20098 (i.p.) effectively suppressed neuronal firing of non-photic cells in the suprachiasmatic nucleus, hippocampus and dorsal lateral geniculate nucleus. Other in vivo studies have also found that melatonin can depress neuronal firing rates of cells in the rat striatum (Castillo Romero et al., 1992), the rat mesencephalic reticular formation (Pazo, 1979), and rabbit cortex (Stankov et al., 1992).

An intracellular recording study has shown that melatonin can lower excitability of guinea pig hippocampal neurons by prolonging IPSPs, raising the threshold for triggering action potentials, and hyperpolarizing membrane potentials (Zeise and Semm, 1985). Our results suggest that S20098, like melatonin, has a widespread inhibitory action on electrical activity of brain cells. It is possible that the effects of S20098 and melatonin on some targets are mediated via the nanomolar affinity binding site for [$^{125}$I]iodomelatonin, the distribution of which is widespread in the Syrian hamster brain (Duncan et al., 1988; Williams et al., 1989; Pickering and Niles, 1990; Ying and Niles, 1989 unpublished data).

Our results are consistent with other evidence that cells in the Syrian hamster suprachiasmatic nucleus are neurophysiologically responsive to melatonin (Stehle et al., 1989; Mason and Rusak, 1990; Yu et al., 1993). A higher proportion of cells in the suprachiasmatic nucleus, whether photically responsive or not, were sensitive to melatonin or S20098 than were cells in the other brain regions we studied. This increased sensitivity may be related to the importance of the suprachiasmatic nucleus as a target for melatonin effects on circadian rhythms in mammals (Cassone et al., 1986, 1987; Duncan et al., 1988, 1989; Weaver et al., 1989; for a review, see Stankov and Reiter, 1990). It is unclear whether modulation by melatonin of neurophysiological responses to light is mediated in part by high-affinity melatonin receptors in the suprachiasmatic nucleus. Such receptors are found in the suprachiasmatic nucleus of Syrian hamsters, but their density varies considerably among subregions of the nucleus and may only minimally overlap the retinorecipient region (Maywood et al., 1995).

Melatonin receptors in different parts of the brain may be structurally heterogeneous. There is evidence from gel chromatography of different molecular weights for melatonin receptors (Barrett et al., 1994). Evidence from biochemical studies indicates that melatonin receptors in some brain regions are coupled to G proteins (Reppert et al., 1994; for a review, see Morgan et al., 1994), but that they are not so coupled in the hippocampus (Barrett et al., 1994) and cortex (Stankov et al., 1993), and melatonin receptors are linked to various second messenger systems (Popova et al., 1995). Furthermore, melatonin binding sites display different affinities for [$^{125}$I]iodomelatonin in different brain regions, including the hypothalamus, midbrain, cerebellum, occipital cortex, hippocampus, pons/medulla, striatum, spinal cord and other brain regions in the male Syrian hamster and in other species (Duncan et al., 1988; Williams et al., 1989; for review, see Stankov and Reiter, 1990, Pickering and Niles, 1990). These findings may help explain why cells in the hippocampus and lateral geniculate region appear to be less responsive to S20098 than cells in the suprachiasmatic nucleus.

The putative melatonin antagonist, S20928, yielded mixed results in studies involving systemic injections. At higher doses, S20928 reduced firing rates of many cells, perhaps by acting as an agonist at melatonin receptors, although this remains speculative. At lower doses, however, S20928 was able to attenuate the effects of melatonin and S20098 on many photically responsive cells in the suprachiasmatic nucleus and intergeniculate leaflet. Metergoline antagonizes the suppression of neural activity induced by serotonin in these brain regions (Ying and Rusak, 1994). However, neither metergoline nor the vehicle used to dissolve S20928 antagonized the effects of melatonin and S20098. These results indicate that at low doses S20928 acts as an antagonist at melatonin receptors found in two major components of the circadian system of Syrian hamsters.

The present electrophysiological study showed that S20928 may function as an agonist and S20928 at low doses as an antagonist for melatonin receptors in the Syrian hamster brain. This conclusion is consistent with previous evidence obtained with other preparations (Redman et al., 1995; Delagrange et al., 1994; Van Reeth et al., 1994). The attenuation by melatonin or S20098 of effects of light on suprachiasmatic nucleus and intergeniculate leaflet neuronal activity indicates that endogenous melatonin and its analogs may be able to modulate photic responses involved in phase shifting and entrainment of the mammalian circadian system.

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