Research report

Apamin, a selective SK potassium channel blocker, suppresses REM sleep without a compensatory rebound

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Abstract

To determine the role of neuronal potassium conductance in rapid-eye-movement (REM)-sleep homeostasis, we have administered small doses of apamin (2–5 ng), a selective blocker of the calcium-dependent SK potassium channel, injected into the lateral ventricle in rats, and characterized the resultant effects on REM-sleep expression. Apamin produces a dose-dependent reduction in REM-sleep expression without an increase in the frequency of attempts to enter REM sleep, suggesting that accumulation of REM-sleep propensity is suppressed. The vast majority (84–95%) of lost REM sleep is not recovered 40 h after apamin administration. These findings suggest that accumulation of REM-sleep propensity is linked to the increased neuronal potassium conductance in nonREM sleep.

Keywords: Sleep function; Sleep homeostasis; Intracerebroventricular; Sleep deprivation; Potassium transport

1. Introduction

Recent findings support the hypothesis that rapid-eye-movement (REM)-sleep propensity accumulates during nonREM sleep rather than during waking [6], and that the timing of each REM-sleep episode is controlled homeostatically by accumulation of REM-sleep propensity to a threshold [4]. These findings corroborate previous studies that demonstrate a dependence of both REM-sleep timing and REM-sleep expression on prior nonREM-sleep expression as opposed to prior waking expression (for review, see [3]). The hypothesis that REM-sleep propensity accumulates during nonREM sleep implies that the function of REM sleep concerns nonREM sleep rather than waking [3].

REM sleep and nonREM sleep are distinguished chiefly by the markedly different patterns of neuronal activity that characterize the two states (reviewed in [26,27]). In the cerebral cortex (reviewed in [19]), thalamus [12,17], and perhaps other brain regions [16], neuronal activity is characterized by a slow oscillatory pattern that underlies the appearance of slow waves in the surface EEG (reviewed in [19]). This pattern is associated with tonic hyperpolarization of the neuronal membrane [8,12,25]. REM sleep, by contrast, is characterized by tonic depolarization and increased activity in most neurons [12,13]. We hypothesize that the physiological property of nonREM sleep that produces a propensity for REM sleep concerns the changes in neuronal membrane conductances that underlie the characteristic neuronal activity patterns of nonREM sleep.

Acetylcholine is the major neurochemical determinant of the changes in neuronal activity patterns associated with changes in sleep/wake states (reviewed in [30]). Increased release of acetylcholine in the cerebral cortex and thalamus during waking and REM sleep tonically depolarizes neurons and decreases input resistance, consequently increasing activity and responsiveness (reviewed in [18]). These effects are mediated predominantly by stimulation of M1 muscarinic receptors. M1 receptor activation blocks at least three K⁺ channels: the voltage-dependent M channel, the Ca-dependent SK channel, and a non-voltage, non-ligand gated K⁺ channel (reviewed in [19]). Other neurotransmitters that may contribute to EEG desynchronization in waking likewise reduce gK⁺ in the cerebral cortex [20]. In sum, the tonic hyperpolarization, reduced activity, and reduced responsiveness of most neurons in nonREM sleep results primarily from increased modulatory gK⁺ in the cell soma and dendrites.
We hypothesize that the accumulation of REM-sleep propensity during nonREM sleep is a consequence of the global increase in gK* that characterizes that state. To test this hypothesis, we have administered apamin, a selective and highly potent blocker of the SK channel (reviewed in [7]), to rats at the beginning of the rest period and characterized the resultant effects on sleep structure. We hypothesize that apamin, by reducing gK* during nonREM sleep, should reduce the rate of accumulation of REM-sleep propensity during nonREM sleep. This would manifest itself by a reduction in REM-sleep expression without subsequent recovery of lost REM sleep. Drive to enter REM sleep, as measured by the frequency of attempts to enter REM sleep [5], should also be reduced.

2. Materials and methods

Adult male Wistar rats, weighing 250–310 g at time of surgery were used in this study. Animals were instrumented with epidural EEG and nuchal EMG electrodes under anesthesia (ketamine 80 mg/kg, xylazine 8 mg/kg, and acepromazine maleate 1.6 mg/kg administered intraperitoneally with intramuscular supplements as necessary). EEG electrodes were #000 stainless-steel screws connected with teflon-coated stainless-steel wires to gold-plated amphenol pins. EEG electrodes were implanted bilaterally over frontal cortex (anterior +1.0 mm, lateral ±2.5 mm from bregma [22]) and parietal cortex (posterior 5.0 mm, lateral ±3.5 mm from bregma). These coordinates were selected to maximize recording of hippocampal theta activity. EMG electrodes were twisted, stainless steel wires in plastic sheaths, threaded through nuchal muscles. Guide cannulae for i.c.v. injections (22 gauge stainless steel tubing, manufactured by Plastics One) were directed at the lateral ventricle (posterior 0.8 mm, lateral 1.5 mm, ventral −3.6 mm from bregma). The guide cannulae were blocked by dummy cannulae until immediately before i.c.v. injections. Animals were given an intramuscular 0.25 ml gentamicin antibiotic injection after surgery and monitored until recovery from anesthesia.

Animals were allowed at least 7 days recovery from surgery and were then acclimated to the recording environment for at least 72 h. Animals were housed individually in transparent Plexiglas cages within electrically grounded Faraday cages. One EEG electrode was used to ground the animal. This set-up allowed EEG recording with negligible movement artifact. The recording room was sound-attenuated, dimly lit (lights on 12 h per day, between 08:00 and 20:00), and maintained at 22–25°C. Animals were given food and water ad libitum.

Animals were cabled via a commutator to a Grass polygraph. Frontal-parietal EEG from one hemisphere was filtered at 0.3 Hz and 35 Hz, digitized at 100 Hz and stored in 10-s epochs on a PC. EEG was Fourier analyzed in 10-s epochs and spectral power was averaged in three frequency bands: delta (0.5–4 Hz), theta (6–9 Hz) and sigma (10–14 Hz). EMG was full-wave rectified, integrated with a 0.5 s time-constant, and stored as one value (0–100) per epoch. Data were collected 24 h per day except for 1 h every 4–8 days for backing up data from the computer's hard disk. Sleep/wake states and transitions from nonREM sleep to REM sleep (NRTs) were scored algorithmically according to the above electrophysiologic indices as described previously [5].

Apamin (SIGMA) was dissolved in artificial cerebrospinal fluid (124 mM NaCl, 26 mM NaHCO3, 3.3 mM KCl, 2.4 mM CaCl2, 1.3 mM MgSO4, and 1.25 mM KH2PO4) and injected in a volume of 2–5 μl at 1 μl/min at the beginning of the light period (08:00 ± 30 min). Injection cannulae (28 gauge stainless-steel tubing, manufactured by Plastics One) were autoclaved prior to use and the apamin solution was passed through a 0.2 μm micropore filter into an autoclaved vial to ensure sterility. Sequential apamin injections in each animal were spaced at least 5 days apart and the order was randomized. Not all animals received all treatments, so statistical tests were not calculated with repeated measures for dose.

Accumulation and discharge of REM-sleep propensity are calculated as follows. In control recordings, the ratio between duration of total nonREM sleep expression and duration of total REM-sleep expression is 4.5:1. Assuming that REM-sleep propensity accumulates at a constant rate during nonREM sleep and is discharged at a constant rate during REM sleep, and that there is no net accumulation or discharge of REM-sleep propensity during 40 h of control recordings, then every 4.5 min of nonREM sleep should result in the accumulation of as much REM-sleep propensity as is discharged during 1 min of REM sleep. For any

![Fig. 1. Dose-response curve for effect of apamin on nonREM-sleep and REM-sleep expression. Plotted are percentage of the first 8 h following apamin administration (mean ± S.E.M.) occupied by nonREM sleep (solid line) and REM sleep (dashed line). The number of animals in each group is given in parentheses at the bottom of the figure. See Results for statistically significant differences.](image-url)
given time period, the net accumulation or discharge of REM-sleep propensity can be calculated by the formula (nonREM sleep/4.5) — REM sleep where positive values indicate accumulation and negative values discharge. The above formula is applied for successive 2-h bins for 40 h following administration of apamin or vehicle to determine whether or not there is compensation for the reduced REM-sleep expression immediately following administration of apamin.

Fig. 2. Effects of 2 ng and 5 ng apamin on sleep variables. Each panel shows data from the first 40 h after administration of apamin (solid line) and vehicle control (dashed line) in 4-h bins. Solid bars at bottom of each panel designate the dark period. Bins for which apamin values differ significantly from vehicle control (Ryan-Einot-Gabriel-Welsch test) are designated by stars immediately above the abscissa ("" P < 0.01, " P < 0.05). Panels A and B plot nonREM-sleep expression as percentage of recording time in each bin. Panels C and D plot REM-sleep expression as percentage of total recording time. Panels E and F plot the number of NRTs per hour of nonREM sleep in each bin. Panels A, C, and E represent data from 5 ng treatments and panels B, D, and E represent data from 2 ng treatments.
All statistical tests were performed using the general linear model (GLM) procedure in SAS, version 6.03. All post-hoc tests employed the Ryan-Einot-Gabriel-Welsh test, a multiple-stage test that controls the type I experiment-wise error rate (see SAS/STAT user’s guide).

3. Results

At the low doses used predominantly in this study, apamin did not induce obvious behavioral abnormalities. Animals exhibited heightened arousal but reduced exploratory behavior. At higher doses (greater than 5 ng), animals displayed resting tremor but no evidence of convulsions and no epileptiform activity in the EEG. There was no appearance of abnormal motor activity during sleep. The EEG indices by which sleep/wake states are scored were not affected by apamin administration at any dose (see Section 2 and [5]).

Apamin produced a dose-dependent decrease in expression of both nonREM sleep and REM sleep during the first 8 h following treatment (Fig. 1). REM-sleep expression was more sensitive to apamin than nonREM-sleep expression. Consequently, lower doses of apamin (e.g., 2 ng) profoundly affected REM-sleep while having little effect on nonREM-sleep. The effects of dose on both nonREM sleep and REM sleep were significant (one-way ANOVAs, n = 5 for each group, P < 0.001). NonREM sleep at 5 ng differs significantly from both 2 ng and vehicle (P < 0.05). REM sleep at both 2 ng and 5 ng differs significantly from vehicle (P < 0.05).

The effects of apamin at 2 ng and 5 ng doses on nonREM-sleep expression, REM-sleep expression, and NRT frequency are shown in Fig. 2. Data are presented in 4-h bins for 40 h after treatment. As 40 h of data are presented in each figure, diurnal variations in all three variables manifest themselves in the vehicle-treated data. The data presented in each panel were statistically analyzed using two-way ANOVAs with repeated measures on the time variable. The significant main and interaction effects of these ANOVAs are summarized in Table 1.

At the 5 ng dose, apamin significantly reduced nonREM-sleep expression for 8 h following treatment, and there was a rebound increase in nonREM-sleep expression during the subsequent active period (Fig. 2A). At the 2 ng dose, apamin produced a small decrease in nonREM-sleep in the first 4 h period and small increases in the subsequent active period (Fig. 2B). Interestingly, the ‘rebound’ increase was considerably greater than the initial loss.

At the 5 ng dose, apamin practically eliminated both REM-sleep expression and NRTs for 12 h after treatment (Fig. 2C, E). The effect of apamin on REM-sleep variables at this dose outlasted the suppression of nonREM sleep by 4–12 h. There was a small rebound increase in REM-sleep in the period 20–24 h after treatment, and the diurnal rhythm of REM-sleep expression in the period 12–40 h after treatment was blunted. At the 2 ng dose, apamin significantly reduced REM-sleep expression 8 h after treatment (Fig. 2D). As with the 5 ng dose, REM-sleep was suppressed when nonREM-sleep expression was unaffected. There was a significant rebound increase in REM sleep during the period 12–24 h after 2 ng apamin. This parallels the increase in nonREM-sleep expression during this period. As with the 5 ng dose, the diurnal rhythm of REM-sleep expression was blunted during the period 12–40 h after treatment. NRT frequency was not significantly affected by 2 ng apamin.

Although there were small rebound increases in REM-sleep expression after both doses of apamin, the vast
majority of the deficit in REM-sleep expression was not recovered. Fig. 3 shows the net loss of REM sleep during the 40 h period following treatment, relative to normal REM-sleep expression (calculated as described in Section 2). After 40 h, the 5 ng dose resulted in 67 min of uncompensated REM sleep and the 2 ng dose in 46 min of uncompensated REM sleep. These values represent 47% and 32% of normal REM-sleep expression during a 24 h period. The final net loss of REM sleep was somewhat less than the peak loss at both doses, indicating that some recovery of lost REM sleep occurred. However, this recovery represents only 5% and 16% of total calculated REM-sleep loss at the 2 ng and 5 ng doses respectively. This recovery was relatively short-lived, and there was no further recovery for the last 12 h of the recording period at either dose. The net loss of REM sleep produced by apamin was statistically analyzed using two-way ANOVAs with repeated measures on the time variable. There were significant main effects of dose ($P < 0.01$) and time ($P < 0.01$), and a significant dose × time interaction ($P < 0.02$). Two-hour bins that differed significantly from vehicle at 2 ng or 5 ng doses are indicated in Fig. 3.

4. Discussion

I.c.v. administration of very small doses of apamin produced two phenomena that indicate suppression of accumulation of REM-sleep propensity. First, REM-sleep expression was suppressed and recovery of lost REM sleep was negligible, resulting in a substantial uncompensated REM-sleep loss 40 h after apamin administration. Second, the frequency of attempts to enter REM sleep (NRTs) was reduced, suggesting that drive to enter REM sleep was not increased, even though REM-sleep expression was suppressed. In sum, REM-sleep expression was reduced without the normal homeostatic responses to REM-sleep deprivation (for review, see [3]).

The substantial amount of uncompensated REM-sleep loss (totaling 32–47% of normal REM-sleep expression during the first 24 h) is perhaps the most telling index of a reduction in the rate of accumulation of REM-sleep propensity. The calculation of this value is based on the assumptions that REM-sleep propensity accumulates during nonREM sleep and that it is accumulated and discharged at constant rates in nonREM sleep and REM sleep. It is worth noting that if we were instead to assume that REM-sleep propensity accumulates during waking (or during both waking and nonREM sleep), net uncompensated REM-sleep loss would be approximately the same, as duration of both nonREM sleep and waking during the 40-h recording period are equivalent in apamin and control treatments. Hence gross (as opposed to net) accumulation of REM-sleep propensity over 40 h is the same in apamin and control treatments in both models.

In the calculations reported above, we assumed that 1 h of REM-sleep propensity accumulates during 4.5 h of nonREM sleep (see Section 2). This value is reasonable in that it reflects the proportion between REM sleep and nonREM sleep in our control recordings. In long-term recordings from rats in a similar recording environment, we elsewhere have reported a nonREM/REM ratio of 4.4:1 [4]. The ratio of 4.5:1 corresponds to REM-sleep expression equaling 18% of total sleep time, which broadly agrees with values obtained from many mammalian species [34].

The existence of uncompensated REM-sleep loss using this calculation is a potent argument for suppression of accumulation of REM-sleep propensity only if REM-sleep loss during non-pharmacological REM-sleep deprivation treatments is substantially recovered. This requirement has been tested using data reported by Van Luijtelaar and Coenen [29] for 72-h REM-sleep deprivations performed by three different methods. We have used the data of Van Luijtelaar and Coenen as a benchmark for comparison because their findings are presented in adequate detail for calculating REM-sleep loss and recovery and because they report the effects of three different methods of REM-sleep deprivation. Using the same assumptions as we have for apamin and applying their reported nonREM/REM ratio of 4.1:1, REM sleep lost during these treatments is recovered at 75–93% efficiency (see Table 2). This result confirms that the calculations procedures we have employed approximately model accumulation and discharge of REM-sleep propensity, and that REM-sleep loss in rats is normally recovered nearly minute-for-minute. In contrast to apamin, other pharmacological manipulations that suppress REM sleep, such as tricyclic antidepressants,

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Data derived from Van Luijtelaar and Coenen [29]. All values expressed in minutes. Amounts of REM sleep lost during REM-sleep deprivation and restored during recovery sleep are determined using a 4:1:1 expected ratio of nonREM sleep to REM sleep (see Section 2), which is the mean proportion between states expressed in baseline recordings preceding all three REM-sleep deprivation methods.
result in a substantial REM-sleep rebound during the withdrawal period (reviewed in [31]).

That accumulation of REM-sleep propensity appears to be suppressed by apamin suggests that accumulation of REM-sleep propensity is associated with increased gK⁺ during nonREM-sleep. Apamin is a highly selective blocker of the SK potassium channel (reviewed in [10]). At higher doses, apamin has deleterious behavioral effects and can be fatal [11], but at the low doses used in this study it appears to produce relatively normal alerting, presumably resulting from tonic depolarization of apamin-sensitive neurons. The SK potassium channel is widely distributed in the central nervous system (CNS), including throughout the cerebral cortex and thalamus [21], and is an important element in the production of synchronized oscillatory neuronal activity during nonREM sleep (reviewed in [19]).

In this study, apamin was administered systemically, producing global reductions in K⁺ flux. This protocol was selected because nonREM sleep is characterized by changes in neuronal activity patterns throughout the CNS. Considerable research effort has been directed at anatomically specific pontine mechanisms of REM-sleep control (reviewed in [27]). However, these data suggest only that the cell groups in question are involved in production of REM-sleep component phenomena. There is no evidence that brainstem sites are preferentially involved in regulation of REM-sleep homeostasis. If REM sleep performs a restorative function in response either to nonREM sleep or to waking, the restorative process very likely proceeds throughout the CNS. There is no a priori reason to assume that the cellular events associated with REM-sleep homeostasis are confined to some regions of the CNS and not others.

The possibility remains that, although apamin may suppress accumulation of REM-sleep propensity, this effect is only indirectly related to the effects of apamin on gK⁺. For example, apamin may stimulate the animal and thereby increase brain temperature. Wehr [33] has hypothesized that the function of REM sleep is to increase brain temperature periodically during sleep. If apamin increases brain temperature, such a hypothesis could account for the uncompensated REM-sleep loss following apamin.

We propose the following, working hypothesis to account for the possible role of gK⁺ in accumulation of REM-sleep propensity. The neuronal K⁺ gradient, which is essential for appropriate electrophysiological responses in neurons, is maintained exclusively by the neuronal Na⁺,K⁺-ATPase (NKA) (reviewed in [28]). Neuronal NKA is insensitive to extracellular K⁺ concentration above 3 mM, the normal minimum level in the CNS [9,24]. The activity of NKA is therefore driven by the intracellular Na⁺ concentration. Na⁺ extrusion and K⁺ infusion by NKA occur in a fixed 3:2 ratio (reviewed in [15]), so preservation of intracellular K⁺ concentration should be dependent on the ratio of Na⁺ flux to K⁺ flux. In non-neuronal cells, the ratio between Na⁺ and K⁺ flux diverges from 1.5 (reviewed in [1]), but this most likely reflects the contribution of other ion pumps, most notably the furosemide-sensitive Na⁺,K⁺-Cl⁻ co-transporter. This pump is present in glia and sympathetic ganglion neurons, but does not appear to be active in CNS neurons [2,14].

Somatic and dendritic K⁺ influx, including Ca²⁺-dependent K⁺ influx, appears to account for the majority of neuronal K⁺ flux, far outweighing axonal K⁺ flux via the action-potential related, delayed rectifier K⁺ channel [23]. Somatic and dendritic gK⁺ is increased in nonREM sleep, presumably resulting in an increase in K⁺ flux relative to Na⁺ flux. In the absence of NKA-independent mechanisms for restoring the K⁺ gradient, this should lead to a progressive reduction in neuronal intracellular K⁺ and concomitant glial KCl uptake (reviewed in [32]).

In REM sleep, neuronal gK⁺ is suppressed by stimulation of M₄ muscarinic cholinergic receptors (reviewed in [18]), thereby increasing the ratio of Na⁺ flux to K⁺ flux. REM sleep may occur to permit replenishment of neuronal intracellular K⁺. This model accounts for the occurrence of REM-sleep episodes at fairly regular intervals throughout sleep and for the rapid increase in REM-sleep drive that occurs when REM-sleep expression is interfered with [6]. This hypothesis is a first attempt to provide a testable physiological basis for REM-sleep propensity.

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