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Noradrenaline acting on α1-adrenoceptor mediates REM sleep deprivation-induced increased membrane potential in rat brain synaptosomes

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Abstract

We hypothesized that one of the functions of REM sleep is to maintain brain excitability and therefore, REM sleep deprivation is likely to modulate neuronal transmembrane potential; however, so far there was no direct evidence to support the claim. In this study a cationic dye, 3,3′-diethylthiacarbocyanine iodide was used to estimate the potential in synaptosomal samples prepared from control and REM sleep deprived rat brains. The activity of Na–K–ATPase that maintains the transmembrane potential was also estimated in the same sample. Further, the roles of noradrenaline and α1-adrenoceptor in mediating the responses were studied both *in vivo* as well as *in vitro*. Rats were REM sleep deprived for 4 days by the classical flower-pot method; large platform and recovery controls were carried out in addition to free-moving control. The fluorescence intensity increased in samples prepared from REM sleep deprived rat brain as compared to control, which reflected synaptosomal depolarization after deprivation. The Na–K–ATPase activity also increased in the same deprived sample. Furthermore, both the effects were mediated by noradrenaline acting on α1-adrenoceptors in the brain. This is the first direct evidence showing that REM sleep deprivation indeed increased neuronal depolarization, which is the likely cause for increased brain excitability, thus supporting our hypothesis and the effect was mediated by noradrenaline acting through the α1-adrenoceptor.

Keywords: $DiSC_2$; Excitability; Membrane depolarization; Na–K–ATPase; Noradrenaline; REM sleep deprivation; Synaptosomes

Rapid eye movement (REM) sleep plays a crucial role in the development and sustenance of the central nervous system as evidenced by its presence across evolution at least from birds to mammals (Amlaner and Ball, 1994; Zepelin, 1994; Siegel et al., 1999) and its decrease with age (Gaudreau et al., 2005). REM sleep is reported to decrease in several diseases including Alzheimer's, Parkinson's (Montplaisir et al., 1995; Gagnon et al., 2002) and in several psychiatric disorders (Vogel, 1999). Its loss has been associated with several signs and symptoms like increased anxiety, aggression, irritability, confusion, loss of concentration (for review see Gulyani et al., 2000) and increased sensitiveness to tactile stimuli where subjects tend to flinch, jump and squeal (Kushida et al., 1989). REM sleep loss has been reported to affect mood; behavior and threshold for electroconvulsive shock in both animal and human subjects (Kushida et al., 1989; Gulyani et al., 2000; Clark, 2005). Recent studies have shown that longer duration of REM sleep deprivation (REMSD) results in morphological changes in neurons (Majumdar and Mallick, 2005) and neuronal death (Biswas et al., 2006; Cordova et al., 2006). Based on these altered behavioral and physiological changes we hypothesized that REMSD alters brain excitability and as a corollary we proposed that one of the functions of REM sleep is to maintain the threshold of neuronal and brain excitability as well as responsiveness (Mallick et al., 1994); however, its mechanism of action was unknown.

A reciprocal relationship exists between the level of excitability and membrane potential in neurons, where the latter is the cause and an estimate of the former. The higher the positivity of the intracellular potential, greater is the excitability level and lower is the threshold of responsiveness of the neuron. As a possible mechanism of action for such changes in excitability we showed that REMSD increases (Gulyani and Mallick, 1993) the activity of the neuronal membrane bound

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enzyme Na–K–ATPase, a key factor that maintains transmembrane potential. On the other hand, REMSD increases the level of noradrenaline (NA) in the brain (for review see Pal et al., 2005). However, no direct study was available to show the effect of REMSD on membrane potential of neurons, which might be responsible for the REMSD-induced altered excitability and its relationship with the increased level of NA. Technical limitation of recording transmembrane potential of the same neuron *in vivo* in freely behaving animals, before and after a reasonable period of REMSD, is a major hurdle for such study. Hence, in this *in vivo* and *in vitro* studies we estimated the membrane potential using a cationic dye and correlated it with Na–K–ATPase activity in the same synaptosomal fraction from the control and REMSD rat brains. Furthermore, we correlated the effect of NA in the presence and absence of its antagonist on those parameters.

1. Methodology

1.1. Materials

Noradrenaline (NA); α1-adrenoceptor antagonist, prazosin (PRZ); β-adrenoceptor antagonist, propranolol (PRN); α2-adrenoceptor agonist, clonidine (CLN); Na–K–ATPase inhibitor, ouabain; mono-cationic fluorescent probe, 3,3-diethylthiacarbocyanine iodide ($DiSC_2$) and dimethyl sulfoxide (DMSO) were procured from Sigma–Aldrich, USA. All other chemicals were of analytical grade.

2. Animals

All the animal experimental protocols were approved by the Institutional Animal Ethics Committee. Male inbred Wistar rats (250–280 g), supplied with food and water ad libitum, maintained at 12/12 h light/dark cycle were used in this study. Standard flower-pot method was used for 4 days REMSD as reported earlier (Gulyani and Mallick, 1993). In brief, the rats were maintained on small (6.5 cm) platform raised over surrounding water. To rule out non-specific effects control rats were maintained under identical conditions for the same period in the same room on raised larger (13 cm) platform (LPC) surrounded by water. Free-moving home cage rats were used as normal control (FMC) for the baseline value. Another control set included rats deprived of REM sleep for 4 days that were then allowed to recover from REM sleep loss for 3 days in their normal home cages, the recovery control (REC). Thus, in each set there was one rat each of FMC, LPC, REMSD and REC and five such sets of studies were carried out. Additionally, in separate series of experiments intraperitoneal injection of PRZ and CLN were done in five different sets, each having one FMC and one REMSD rats. For *in vitro* experiments synaptosomes prepared from the FMC rat brain were treated with NA alone or in the presence of either PRZ or PRN. Since PRN did not prevent the NA-induced effects in *in vitro* studies, it was not used for the *in vivo* studies. Thus, every effort was made to minimize the use of number of rats and finally, data from 45 rats are presented in the study.

2.1. Synaptosome preparation

The deprived as well as the control rats were decapitated after cervical dislocation. The brains were quickly removed and homogenized in 10 volumes of ice-cold buffer containing 0.32 M sucrose and 12 mM Tris at pH 7.4 for synaptosome preparation from the whole brain (Mallick and Adya, 1999). In brief, the brain homogenate was centrifuged for 5 min at $3000 \times g$ and the supernatant was further centrifuged at $11{,}000 \times g$ for 20 min. The pellet obtained was suspended in 1 ml of the homogenizing buffer, loaded onto a preformed sucrose density gradient of 1.2 M and 0.8 M and ultracentrifuged in a swing-out rotor at $100{,}000 \times g$ for 2 h. The band obtained at the interface of 1.2 M and 0.8 M sucrose was taken as synaptosome, which was divided into two parts. One part was re-suspended in the homogenizing buffer while the other part was re-suspended in the HEPES buffer containing 140 mM NaCl, 5 mM KCl, 5 mM $NaHCO_3$, 1 mM $MgCl_2{\cdot}6H_2O$, 1.2 mM Na_2HPO_4, 10 mM glucose, 20 mM HEPES at pH 7.4. The former was used for assaying Na–K–ATPase activity while the latter for synaptosomal membrane potential study. The protein concentration in the synaptosomes was estimated by the Lowry's method (1951) using bovine serum albumin as standard.

2.2. Modulation of membrane potential and Na–K–ATPase activity by NA

The studies were conducted both *in vivo* and *in vitro* conditions. Both membrane potential as well as Na–K–ATPase activities were estimated from the same synaptosomal fraction of FMC, LPC, REMSD and REC rat brains. For the *in vivo* experiments either PRZ (4 mg/kg) or CLN (0.1 mg/kg) was injected (i.p.) into both the FMC and REM sleep deprived rats 8 h before sacrifice. For the *in vitro* studies synaptosomes prepared from the FMC rat whole brain were incubated with NA (100 μM) alone or in the presence of either PRZ (50 μM) or PRN (50 μM) and membrane potential as well as Na–K–ATPase activities estimated.

2.3. Estimation of membrane potential

$DiSC_2$ is a membrane permeable mono-cationic fluorescent dye, which accumulates within the synaptosomes that resembles the inside of a neuron and therefore is relatively negative (compared to the extracellular compartment). It has been convincingly shown that $DiSC_2$ movement inside the cells is directly proportional to the intracellular negativity (Wang et al., 2001). The anions inside the synaptosomes bind to $DiSC_2$ and consequently quench its fluorescence. Hence, greater the intracellular negativity more was the $DiSC_2$ quenching, resulting in reduced net fluorescence intensity. Thus, intensity of fluorescence was inversely proportional to the intracellular anion concentration and as a corollary increased $DiSC_2$-fluorescence intensity meant membrane depolarization (Waggoner, 1976; Hare and Atchison, 1992). Therefore synaptosomal (reflection of intracellular) membrane potential was estimated