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REM Sleep Deprivation Decreases the Grooming and Shaking Behaviour Induced by Enkephalinase Inhibitor or Opiate Withdrawal

O. E. UKPONMWAN, A. L. v. D. POEL-HEISTERKAMP AND M. R. DZOLJIC[1]

Department of Pharmacology, Faculty of Medicine, Erasmus University Rotterdam, P.O. Box 1738 3000 DR Rotterdam, The Netherlands

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UKPONMWAN, O. E., A. L. v. D. POEL-HEISTERKAMP AND M. R. DZOLJIC. *REM sleep deprivation decreases the grooming and shaking behaviour induced by enkephalinase inhibitor or opiate withdrawal.* PHARMACOL BIOCHEM BEHAV **23**(3) 385–389, 1985.—Intraventricular administration of enkephalinase inhibitor, phosphoramidon (1×10^{-8}–5.6×10^{-7} moles ICV) induced a behavioural syndrome consisting of excessive grooming with the body scratching as the most prominent symptom and wet-dog-shakes (WDS). The frequency of the phosphoramidon-induced WDS and body scratching were decreased by the pretreatment with the opiate receptor blocking agent, naltrexone (2.9×10^{-6} moles/kg IP). Both the phosphoramidon-induced WDS in naive rats and naloxone-precipitated withdrawal WDS were decreased in REM sleep deprived rats compared with animals allowed normal sleep (control and stress groups). The results are discussed in light of a possible functional insufficiency of endorphinergic system during REMSD. It has been suggested that this insufficiency might be a background to the increased neuronal excitability during REMSD.

REM sleep deprivation Enkephalinase inhibition Phosphoramidon Opiate withdrawal

SEVERAL endogenous substances including opioid peptides have been demonstrated to induce grooming and wet-dog-shakes [5, 6, 9, 12].

Recently, we demonstrated that the administration of the enkephalinase inhibitor, phosphoramidon, induced behaviours such as grooming and wet-dog-shakes (WDS) [28]. These behavioural phenomena, which can be induced by various drugs in naive animals, are part of the morphine withdrawal syndrome. There are indications that both grooming and WDS may share a common neural mechanism [10].

It has been demonstrated that inhibition of protein synthesis reduced the severity of opiate withdrawal phenomena [19]. In addition it is known that REM sleep deprivation (REMSD) can decrease protein syntehsis [30]. REMSD also inhibited morphine induced analgesia [38]. These data suggest that alterations in REM sleep can modulate both protein synthesis and pharmacological effects of opiate substances. Therefore, we analyzed the relationship between REMSD and grooming and/or shaking behaviour induced by enkephalinase inhibitor phosphoramidon in naive rats and naloxone-precipitated withdrawal in the opiate-dependent rats.

METHOD

Adult, male Wistar rats (100–125 g) housed in transparent plastic cages in a constant environment room with a light-dark cycle 14:10 (light phase 07.00–21.00) were used.

Intracerebroventricular (ICV) Administration of Drug Solutions

For ICV administration of drugs a stainless steel guide cannula was stereotaxically directed 1 mm above the lateral ventricle. Drug solutions (maximum volume 2 μl) were injected into the lateral ventricle with a gauge 30 needle, attached to a Hamilton microsyringe by polyethylene (PE) tubing. The length of the needle was made such that it protruded 1 mm into the lateral ventricle. The injection was made over 10 sec and the needle maintained in position for an additional 10 sec. Correct ventricular cannulation was verified before and after each experiment using a modification of the technique previously described by Paakkari [24]. In this procedure a PE tubing is attached to the injection needle and filled with artificial cerebrospinal fluid (CSF) or saline. To test the correct placement of ICV cannula during surgery, the tubing is raised above the head of the animal on the stereotaxic apparatus, and a rapid inflow of saline denotes a correct placement of cannula. The cannula was moved only in a downward direction to avoid the possible false positive effect due to an upward movement of the cannula after the first unsuccessful cannulation attempt [14].

REM Sleep Deprivation (REMSD)

REMSD was carried out according to the conventional 'flower pot' technique previously described [21]. In this pro-

[1]Requests for reprints should be addressed to M. R. Dzoljic.

cedure, rats were placed on platforms (14 cm²/100 g rat) surrounded by water, such that the water level was 0.5–1.0 cm below the platform. Rats made morphine-dependent were placed on platform and deprived of REM sleep from day 7–11 (96 hr) of morphinization. During this period animals received the normal doses of morphine for these days. Forty-five to 60 min after discontinuation of REMSD the animals were injected with the enkephalinase inhibitor phosphoramidon. In this period of time the animals were kept awake manually. The behavioural changes were scored during the following 30 min.

Control for the Unspecific Stress Factors Associated With the 'Flower Pot' Technique

In order to control for the known stress factor (dampness, isolation and immobilisation) associated with the 'flower pot' technique rats were placed on platforms large enough (60 cm²/100 g rat) for them to curl up and have normal sleep. The large platform can simulate the chronic stress condition associated with REMSD without affecting the REM sleep level after 96 hr [20,21].

Phosphoramidon-Induced Behaviour

Behavioural observation and scoring were carried out on each individual rat housed singly in Plexiglas cages (40×20×15 cm) containing sawdust. Wet-dog-shakes consisting of paroxysmal shudder of the whole body along the spinal axis were registered and quantified. The body scratching (BS) episode is defined as head or body scratches followed immediately by the licking paw used in scratching.

Induction of Morphine Dependence

Animals were made dependent on morphine according to the repeated injection procedure previously described [32]. In this method rats were given two intraperitoneal injections of morphine daily (at 07.30 and 15.30 hr). The dosage schedule was as follows: Days 1 and 2 (7×10^{-5} moles/kg/day); Days 3 and 4 (14×10^{-5} moles/kg/day); Days 5 and 6 (28×10^{-5} moles/kg/day) and Days 7–11 (56×10^{-5} moles/kg/day).

Precipitation of Morphine Withdrawal Shaking Behaviour

Abstinential behaviour in morphine dependent animals was provoked by injecting naloxone (3.1×10^{-6} moles/kg IP) three hours after the last dose of morphine. Prior to receiving naloxone treatment each rat was allowed a habituation period of 30–60 min in the observation area. Naloxone-precipitated WDS in morphine-dependent rats and phosphoramidon-induced behavioural phenomena in naive animals were studied in the following three groups of rats: (a) *control group*—these rats were housed singly in home cages throughout the experimental procedure and allowed spontaneous amounts of sleep; (b) *REM sleep deprived group*—these rats were submitted ot 96 hr continuous REM sleep deprivation; (c) *stress group*—these animals were chronically stressed for 96 hr.

Drugs

The following drugs were used: morphine hydrochloride (Merck), naloxone hydrochloride (Endo Lab) and naltrexone hydrochloride (Endo Lab) were administered dissolved in physiological saline. Phosphoramidon (Peninsula Lab) was dissolved in CSF prepared fresh and administered ICV.

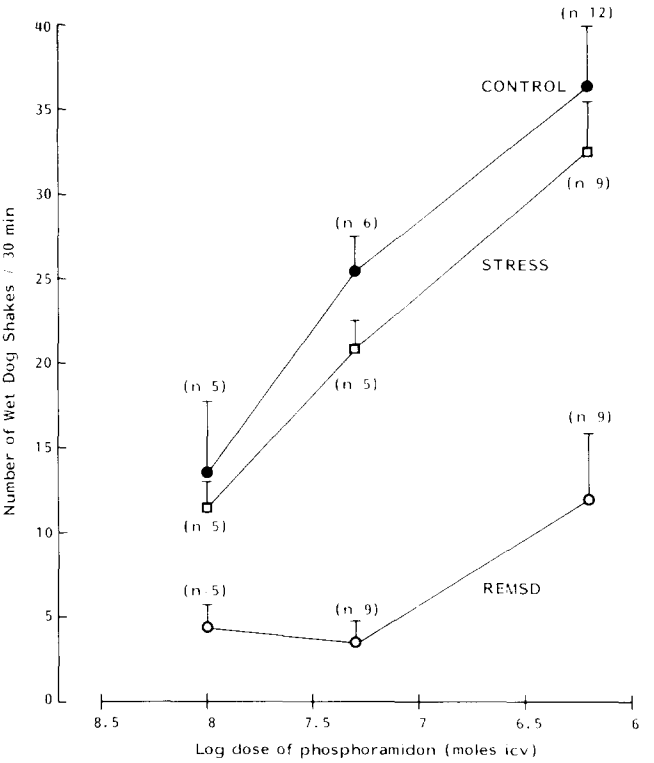

FIG. 1. The phosphoramidon (1×10^{-8}–5.6×10^{-7} moles ICV)-induced wet-dog-shakes (WDS). Each point is mean±S.E.M. The number of rats per dose of phosphoramidon is stated in parentheses. Note that the phosphoramidon-induced WDS was significantly lowered in REMSD rats compared with control or stressed animals.

Data Analysis

The results were analyzed using the Kruskal-Wallis one-way ANOVA. The statistical difference between two groups of treatments were carried out using a two-tailed Mann Whitney U test, except when indicated in text.

RESULTS

Effects of REMSD on the Behavioural Syndrome Induced by Enkephalinase Inhibition

The intracerebroventricular administration of the enkephalinase inhibitor phosphoramidon (1×10^{-8}–5.6×10^{-7} moles ICV) induced a behavioural syndrome consisting of excessive grooming (as measured by body scratching) and wet-dog-shakes (WDS) in all three groups of animals (control, REMSD and stressed). These symptoms appeared within 5 min after phosphoramidon administration and were still observed after 240 min. The Kruskal-Wallis one-way ANOVA showed a significant difference in the phosphoramidon-induced WDS across the groups ($H=106.21$, $NDF=8$, $p<0.001$). The frequency of the phosphoramidon-induced WDS was dose-related in both control ($p<0.05$) and stressed ($p<0.02$) groups of animals (Fig. 1). However, the frequency of the phosphoramidon induced WDS in stressed rats was not significantly different from control animals (Fig.

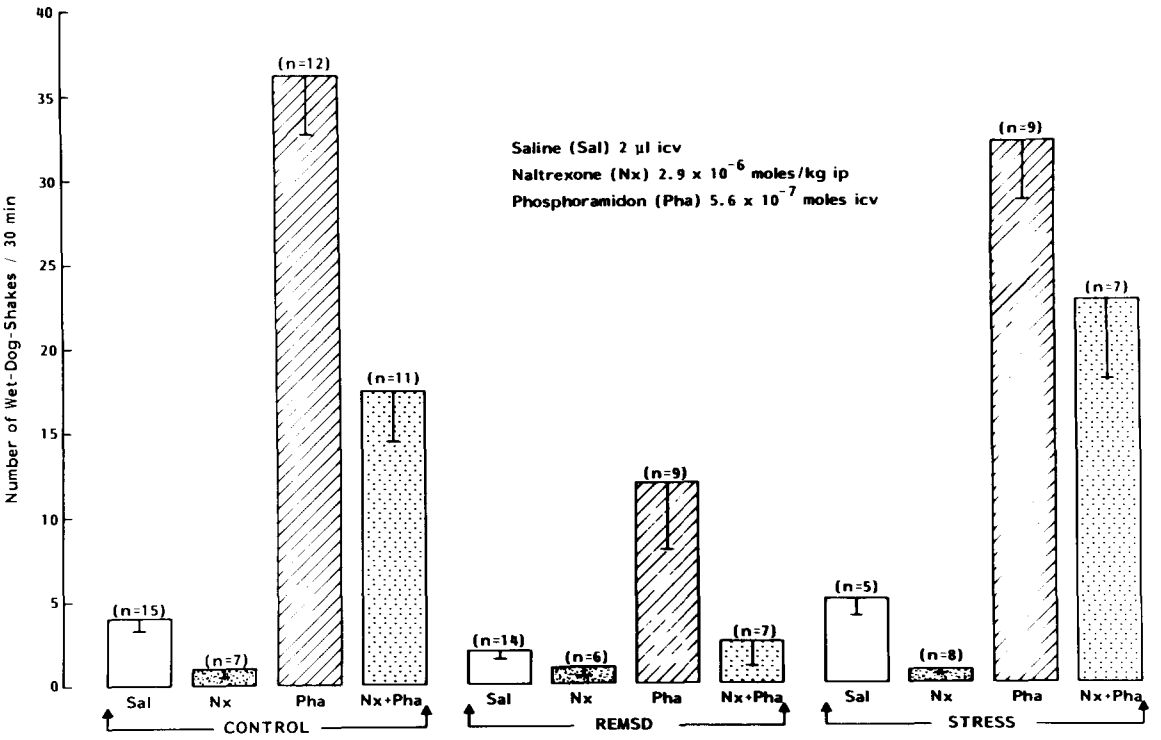

FIG. 2. Effect of naltrexone (2.9×10^{-6} moles/kg IP) on phosphoramidon (5.6×10^{-7} moles ICV)-induced WDS. Each bar is mean±S.E.M. The number of animals per treatment group is indicated in parentheses. Note that pretreatment with naltrexone significantly decreased phosphoramidon-induced WDS.

1, $p>0.05$). REMSD significantly decreased the WDS induced by the three doses of phosphoramidon (Fig. 1, $p<0.02$, $p<0.002$, $p<0.002$ respectively for increasing doses). The frequency of BS in the REM sleep deprived rats was significantly less intensive compared with control and stressed animals (Fig. 3, $p<0.02$). There was no significant difference in mean BS between control and stressed animals (Fig. 3, $p>0.10$).

Naltrexone (2.9×10^{-6} moles/kg IP, 10 min prior) significantly decreased the phosphoramidon-induced WDS in control ($p<0.002$), REMSD and stressed animals ($p<0.05$, Duncan New Multiple range test) (Fig. 2). In the control rats the phosphoramidon-induced BS were significantly less frequent after pretreatment with naltrexone (92.8±23.6, n=11) compared with saline pretreated animals (201.8±29, n=12, $p<0.05$).

Effect of REMSD on Opiate Withdrawal WDS

Naloxone (3.1×10^{-6} moles/kg IP) precipitated WDS in morphine-dependent rats in control, REM sleep deprived and stressed groups of animals. The Kruskal-Wallis one-way ANOVA showed a significant difference in the withdrawal WDS across the groups (Fig. 4, H=16.12, NDF=2, $p<0.001$). The frequency of the precipitated WDS was significantly more pronounced in animals allowed to sleep normally (control and stressed groups) than in the REM sleep deprived rats (Fig. 4, $p<0.002$). However, the intensity of such induced WDS in control or stressed animals was not significantly different (Fig. 4, $p>0.2$). Body scratchings in naloxone-treated morphine dependent rats were few and irregular and therefore omitted from further detailed quantitative evaluation.

DISCUSSION

The results of this study showed that the WDS and grooming induced by the enkephalinase inhibitor, phosphoramidon, were inhibited by naltrexone, which might indicate an involvement of opiate receptor(s). This is consistent with the fact that enkephalinase inhibition can activate opiate receptors by blocking the biotransformation of endogenously released opioid peptides [16,25]. In addition, the WDS-induced by ICV administration of enkephalins were attenuated by opiate antagonists [2, 5, 6, 13].

Grooming behaviour has also been observed after low doses of morphine [29]. Taken together, these data might suggest that WDS and grooming induced by phosphoramidon or opioid substances share a common mechanism.

The biological significance of WDS and grooming induced by different chemical compounds is not clear. Some data suggest that WDS are indicative of arousal [10], whereas grooming might be a 'de-arousing' homeostatic mechanism [17]. In addition, it has been demonstrated that opioid peptides facilitate arousal [37] and in higher doses induced an electrophysiological and behavioural phenomena similar to epilepsy [7,8]. Thus the excessive grooming observed in our

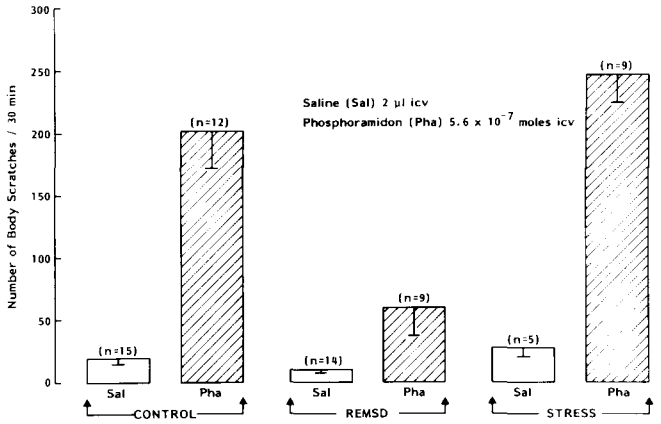

FIG. 3. Effect of REMSD on phosphoramidon (5.6×10^{-7} moles ICV)-induced body scratches. Each bar is mean±S.E.M. The number of rats per treatment group is stated in parentheses. Note that the intense body scratches induced by phosphoramidon in control and stressed animals were significantly decreased by REMSD.

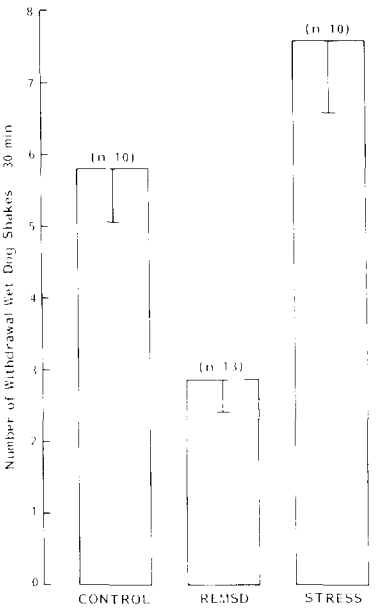

FIG. 4. Effect of REMSD on naloxone (3.1×10^{-6} moles/kg IP)-precipitated withdrawal WDS. Each bar is mean±S.E.M. The number of rats per group is indicated in parentheses. Note that the total frequency of withdrawal WDS was significantly less in REMSD rats compared with control ($p<0.01$) and stressed ($p<0.002$) animals.

experiment might be a response to the phosphoramidon-induced arousal, manifested as WDS.

However, the most important aspect of this report is the fact that REMSD suppressed the WDS and grooming induced by enkephalinase inhibition in naive rats. Why REMSD decreased these behaviours in rats is not clear. It could, however, be suggested that REM sleep deprived animals might have limited availability of opioid peptides and hence the WDS and grooming precipitated by phosphoramidon could be less pronounced. Although there is no direct biochemical evidence for insufficiency of the enkephalinergic system during REMSD this possibility could be considered since it is known that REMSD is associated with the inhibition of protein synthesis [30]. The concept of a functional insufficiency in the enkephalinergic/endorphinergic system in REM sleep deprived animals receives further support from the fact that REMSD abolished the antinociceptive effects of morphine and phosphoramidon [38].

This hypothesis of a functional insufficiency in this opioid system might explain the increased neuronal excitability during REMSD [4] since it is known that endorphins exhibit tonic inhibitory effects on the release of excitatory transmitters [23]. However, additional experiments are required to clarify whether this mechanism might be involved in REMSD-precipitated seizures and the therapeutic effect of REMSD in some types of endogenous depression.

A second important finding of this study is that REMSD inhibited abstinential WDS. The mechanism of opiate addiction/withdrawal is complex and probably involves alteration in several neurotransmitter/neuromodulator systems. However, the known changes in classical transmitters during REMSD can not account for the decrease in naloxone-precipitated withdrawal WDS in REM sleep deprived animals. For example, REMSD increased the functional activity of the dopaminergic system [36], but did not alter the adrenergic system [31]. However, substances which block these systems inhibited withdrawal WDS [18,34]. Furthermore, the known changes in brain serotonin metabolism during REMSD [33] probably play no role in the inhibition of abstinential WDS in REM sleep deprived rats, since the alteration of the serotoninergic system had no clear effect on WDS induced by morphine withdrawal [1]. It is also known that drugs which stimulate central muscarinic receptors inhibited the shaking response [39], whereas REMSD decreased the acetylcholine content of the brain [3,35].

Although some high energy phosphates can antagonize the effects of morphine, there is no evidence that concentrations of AMP, ADP and ATP are significantly altered by REMSD [11,22].

Therefore, an alternative explanation for the inhibitory effect of REMSD on morphine withdrawal WDS should be considered. Namely, it is known that during development of morphine dependence there is an increase of the synthesis of secretory proteins in the brain regions (pons-medulla and stratum-septum) [27], which are particularly rich in opiate receptors [26] and functionally involved in opiate dependence [15]. It has also been demonstrated that REMSD decreased protein synthesis in the cerebral and brain stem fractions [30]. Inhibition of protein synthesis can decrease opiate withdrawal phenomena [19]. Thus the decrease of protein synthesis during REMSD might explain the inhibitory effect of REMSD on withdrawal WDS.

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