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Effects of REM Sleep Deprivation on Central α_1- and β-Adrenoceptors in Rat Brain

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MOGILNICKA, E., B. PRZEWŁOCKA, E. L. J. M. VAN LUIJTELAAR, V. KLIMEK AND A. M. L. COENEN. *Effects of REM sleep deprivation on central α_1- and β-adrenoceptors in rat brain.* PHARMACOL BIOCHEM BEHAV 25(2) 329–332, 1986.—In the present experiment the effects of 'rapid-eye-movement' sleep deprivation (REMd) on cortical α_1- and β-adrenoceptor binding sites in the rat brain were investigated. REMd was induced for 72 hr in two different ways: by the platform and the pendulum technique. In addition, three control groups were run. Determination of α_1- and β-adrenoceptor sites in the cortex was done by ^{3}H-prazosin and ^{3}H-dihydroalprenolol binding studies, respectively. Both REM sleep deprived groups showed a small but significant decrease in the number of β-adrenoceptor sites along with a small increase in affinity. On the other hand, α_1-adrenoceptor binding and affinity were not changed. These results agree with the effects of tricyclic antidepressant drug treatment. Common effects of REMd and tricyclic drugs are discussed in terms of modulation of tonic arousal processes.

REM sleep deprivation Platform technique Pendulum technique ^{3}H-prazosin binding ^{3}H-DHA binding α_1-Adrenoceptors β-Adrenoceptors

BESIDES anti-depressant drug treatment, deprivation of 'rapid-eye-movement' sleep (REMd) is also effective in the treatment of endogenous depressive patients [31]. In order to determine possible underlying common mechanisms behind these two treatments, it seems imperative to compare the effects of both anti-depressant drugs and REMd in animal models. Aside from the clinical results, there is also some agreement between anti-depressant drug treatment and REMd on behavioural and biochemical measures. Clonidine-induced sedation and imipramine binding are both affected by anti-depressants and REMd [4, 16, 17, 20]. Furthermore, the immobility duration, as determined in Porsolt's forced swim test, can be reduced by anti-depressants and by REMd [18,28].

Wojcik and Radulovacki [32] and Schildkraut and Hartmann [22] found enhanced noradrenergic transmission after REMd. It is commonly known that this is also the case following administration of desipramine (e.g., [23]). Therefore, Radulovacki and Micovic [19] suggested that both desipramine treatment and REMd produced similar effects on central noradrenergic synapses. A long-term enhanced noradrenergic transmission is related to the finding that chronic anti-depressant drugs give rise to a reduction in the density of β-adrenoceptor sites [2,32]; an effect that is interestingly enough equivalent to that of REMd [17], although less convincing [19] and neutral results were also reported [1].

In contrast to a down-regulation of β-adrenoceptors, an up-regulation of α_1-receptor activity is sometimes found as a result of chronic administration of anti-depressant drugs [4,29], though conflicting results have appeared [11,21]. With respect to the effects of REMd on this type of receptor activity no data are available.

REMd in the above-mentioned studies is, as is generally done, performed with the classical small platform technique [12], while a larger platform is used to control for possible adverse effects. Numerous effects of REMd with the platform technique can be found on several parameters (see e.g., [7,30]). However, it is uncertain whether the obtained changes are induced by REMd per se, or are induced by non-specific treatment effects of the platform technique [26–28, 30]. Furthermore, it was recently shown that an alternative instrumental REMd technique, the pendulum technique [25], produced differential effects on several behavioural and electrophysiological parameters (e.g., [26–28]. Nowadays, it is still not completely clear which of the techniques is more valid. Nevertheless, the use of two REMd techniques will yield at least a common REMd effect, while non-specific effects will differ.

Given the similarities in effects in many behavioural and pharmacological tests, of both chronically applied anti-depressant drugs and REMd, it seemed interesting to test how REMd affects the ^{3}H-prazosin and ^{3}H-dihydroalprenolol

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(^{3}H-DHA) binding to α_1- and β-adrenoceptors in the cortex of the rat brain.

METHOD

Subjects were naive male Wistar rats from the Wu:Cpb strain [8], which were housed in triplicates in standard macrolon cages with free access to food and water. Rats were kept in a temperature-controlled room (20–22°C) and maintained on a 12-12 hr light-dark cycle, with white lights on at 22.00 hr throughout the experiment. One week before the experiment the animals were housed individually and handled on five successive days. At the same time animals assigned to pendulum conditions were transferred to specially adapted cages. Immediately prior to experimentation the mean body weight was 285 g (range 248–330 g), corresponding to an age of about 3 months. REMd was achieved by the platform or the pendulum technique. The platform technique consists of either a small (diameter 6.2 cm) or, for control purposes, a large (diameter 12.8 cm) platform surrounded by water. Food and water were freely available. The pendulum technique was described in detail elsewhere [25], and included an electrophysiological evaluation. In brief, rats, in their home cages, are placed in a slowly moving swing which produces regular postural imbalance and subsequent awakening at the two extremes of oscillation. This permits brief periods of slow-wave sleep but prevents REM sleep. In the pendulum control condition an identical swing was adjusted so as to obviate postural imbalance. In total, five groups were used: (1) a 'home cage control' group, (2) a 'pendulum experimental' group in which rats were REM sleep deprived for 72 hr, (3) a corresponding 'pendulum control' group, (4) a 'platform experimental' group in which rats were deprived of REM sleep for 72 hr by placing them on a small platform, (5) a corresponding 'platform control' group in which large platforms were used. All treatments started at the onset of dark.

Rats were killed within 15 sec after completing REMd, their brains quickly removed and the neocortex dissected and rapidly frozen. For ^{3}H-prazosin and ^{3}H-DHA binding studies, the neocortex was homogenized in 20 vol. (w/v) of an ice-cold Tris HCl buffer (50 mM, pH 7.4) using a Polytron homogenizer. The homogenates were centrifuged at 25.000 g for 10 min; pellets were rehomogenized in another portion of buffer and then centrifuged. The final pellets were resuspended in 140 vol. (w/v) of Tris HCl buffer (50 mM, pH 7.4). To 1.4 ml of membrane suspensions were added ^{3}H-prazosin (NEN, spec. act. 17.4 Ci/mmol) or ^{3}H-DHA (NEN, spec. act. 55.4 Ci/mmol) in a volume of 100 μl, after which the samples were incubated at 25°C for 25 min. Afterwards the total incubation volume of 2 ml was poured over glass filters (Whatman GF/C) and rinsed three times with 5 ml of the ice-cold buffer (Tris HCl, pH 7.4). Tritium was estimated by a conventional liquid scintillation counter. The specific binding of ^{3}H-prazosin was defined as the difference between the amount bound in the presence and absence of 1 μM of phentolamine, while the ^{3}H-DHA binding was defined as the difference between that which occurred in the presence and absence of 1 μM (−)alprenolol.

The specific binding of ^{3}H-prazosin was, on the average, about 70% of the total binding, while the specific binding of ^{3}H-DHA was, on the average, about 75% of the total binding. The ^{3}H-prazosin and ^{3}H-DHA concentration, ranging from 0.025 nM to 1.0 nM and from 0.05 mM to 1.5 nM, respectively, were used for Scatchard plots. B_{max} and K_D values were calculated individually for each rat by the Scatchard analysis, using 6–7 concentrations of ^{3}H-prazosin or ^{3}H-DHA and performing the assay in duplicates.

RESULTS

Table 1 presents the effects of REMd or control treatment applied for 72 hr on the density of the binding sites (B_{max}) and the affinity constant (K_D) for the ^{3}H-prazosin and ^{3}H-DHA binding in the rat cerebral cortex.

Planned orthogonal comparisons [10] showed a significant reduction due to REMd on the maximal number of ^{3}H-DHA binding sites, the B_{max} when the two REMd groups were compared with their controls, $F(1,25)=8.25$, $p<0.01$. The K_D values were also lower for the two experimental groups compared to their controls, $F(1,25)=10.55$, $p<0.01$. which means that the affinity of the binding sites is increased as a consequence of REMd. Post-hoc comparisons (Duncan's multiple range tests [6]) showed that the K_D values of the two control groups differed from the home cage control group ($p<0.05$). On the other hand, post-hocs for the B_{max} of the ^{3}H-DHA showed no differences between the three control groups. With respect to the ^{3}H-prazosin binding, there was no effect of REMd on K_D and on B_{max}. A significant difference between the pendulum control and large platform control group was detected, $F(1,23)=7.03$, $p<0.01$, on the B_{max} without affecting the K_D. No other (post-hoc) differences were significant.

DISCUSSION

Taking the three literature reports [1, 17, 19] with respect to the effects of REMd on β-adrenoceptor density together with this report, the main conclusion must be that REMd leads to a small, but significant, reduction of β-adrenoceptor density. A small β-down-regulation, however, might give rise to marked effects on other systems [24]. The fact that in the present study the reduction occurs with different methods of REMd—with differential non-specific effects—testifies that the change in β-adrenoceptors is the result of REMd per se. This is also true for the K_D values. The decreased values showed that the affinity of the β-receptors is enhanced. The diminished β-adrenoceptor density together with the increased affinity points into the direction of compensatory mechanisms.

A point difficult to explain is that the B_{max} of the home cage group is the same as the two treated control groups, while the K_D of the first group is equal to the experimental (REMd) groups. It is, however, not impossible that treatment gives rise to a specific enhancement of the K_D and that REMd counteracts this effect. As a result of REMd, the α_1-adrenoceptor density remained unchanged; neither did the affinity for ^{3}H-prazosin change. The only finding was that the large platform group differed significantly in α_1-receptor density from the pendulum control group; this finding is difficult to interpret but questions the adequacy of the large platform as a control.

There are reports demonstrating an increase in the α_1-adrenoceptor density in the cerebral cortex of the rat after chronically given antidepressant drugs [3,29]. It should, however, be added that papers from other laboratories have not reported any changes in α_1-adrenoceptors [11,21]. The lack on alterations in this binding may confirm the opinion that α_1-adrenoceptors are considerably more resistant to modulation than β-adrenoceptors [5]. It may also be due to the fact that ^{3}H-prazosin—the antagonist ligand—was used. Evidence has been accumulated that changes in agonist, as

TABLE 1

ADRENOCEPTOR ALTERATIONS IN THE RAT CEREBRAL CORTICAL MEMBRANES AFTER 72 HR REM SLEEP DEPRIVATION OR CONTROL TREATMENT

	^{3}H-Prazosin			^{3}H-DHA		
	n	B_{max} (pmol/g wet weight)	K_D (nM)	n	B_{max} (pmol/g wet weight)	K_D (nM)
Home cage	7	8.36 ± 0.20	0.058 ± 0.005	7	5.90 ± 0.19	0.53 ± 0.04
Pendulum Experimental	7	8.28 ± 0.17	0.055 ± 0.005	7	5.08 ± 0.24	0.52 ± 0.04
Platform Experimental	7	8.31 ± 0.18	0.050 ± 0.003	7	5.36 ± 0.24	0.58 ± 0.03
Pendulum Control	4	8.61 ± 0.35	0.040 ± 0.003	4	6.11 ± 0.21	0.64 ± 0.02
Platform Control	4	7.73 ± 0.07	0.049 ± 0.007	5	5.69 ± 0.04	0.70 ± 0.03

Mean and S.E.M. are given, n=number of Scatchard plots.

The dissociation affinity constants K_D (nM) and the maximal binding B_{max} (pmol/g wet weight) were obtained by Scatchard analysis. Scatchard plots, each of 6–7 points, were determined in duplicates, using concentration ranges from 0.025 to 1 nM for ^{3}H-prazosin and from 0.05 to 1.5 nM for ^{3}H-DHA.

a: groups are significantly different at 0.01 level; b: groups are significantly different at 0.05 level.

compared to antagonist binding, may provide a more sensitive index of physiologically relevant receptor changes. However, α_1-adrenoceptor binding, measured with the antagonist ligands is generally unaltered in the brain after chronic anti-depressants [11,21]. Recent studies of Menkes *et al.* [15] showed that, following treatment with tricyclic antidepressants, central α_1-adrenoceptors exhibit supersensitivity to α_1-agonists which is consistent with previous physiological [14] and behavioural [13] studies.

On the other hand, it is now well established that chronic treatment of rats with tricyclic anti-depressant agents decreases the number of β-adrenoceptors. REMd also causes a reduction in the receptor density. Therefore, β-down regulation can be regarded as a common feature of chronic anti-depressant treatment and REMd. It is worthy to note that REMd as well as antidepressant drug treatment lead to an increase of noradrenergic turnover [22,32]. Together with β-down regulation (an effect that may be a result of this increase [2]), these two common effects of tricyclic antidepressants and REMd are often considered as important elements in the clinical efficacy of anti-depressant treatments [2, 19, 23].

Enhancement of noradrenergic activity is often interpreted as having activating properties. In this respect, it is striking that Van Hulzen and Coenen [27] found a decrease in rats' visual evoked potentials following REMd, which decrease in neural excitability was interpreted as an increase in tonic arousal. Friedman and Meares [9] found in depressive patients that the amplitude of evoked potentials were larger during depression that after recovery, either as a result of anti-depressant or placebo treatment. This suggests that recovery is correlated with increased tonic arousal, as manifested by reduced amplitudes of evoked potentials.

In short, tricyclic anti-depressants and REMd have β-down regulation and enhanced noradrenergic turnover in common. This may be correlated with a decrease in amplitude of evoked potentials, suggesting an increase in tonic arousal.

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