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Dopamine Metabolism and Receptor Sensitivity in Rat Brain After REM Sleep Deprivation

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FARBER, J., J. D. MILLER, K. A. CRAWFORD AND B. A. McMILLEN. *Dopamine metabolism and receptor sensitivity in rat brain after REM sleep deprivation.* PHARMACOL BIOCHEM BEHAV 18(4) 509–513, 1983.—Different dopaminergic mechanisms that could explain behavioral supersensitivity to amphetamine or apomorphine in REM-deprived rats were examined. Four days of REM sleep deprivation induced a highly significant elevation in striatal DOPAC relative to normal controls, but not to stress controls. DOPAC levels in frontal cortex were not affected in any of the groups. Post synaptic D_2 receptor number (B_{max}) and affinity (K_d) were unchanged in both terminal regions. Similarly, no changes in pre-synaptic receptor sensitivity (apomorphine-induced inhibition of tyrosine hydroxylase) occurred in striatum. A stress control group exhibited no changes in any of the biochemical measures in comparison with either the REM deprived group or unstressed controls. Thus, the enhanced response to dopamine agonists reported previously is not due to altered dopamine receptor sensitivity. Alternative hypotheses to explain enhanced responses to direct and indirect acting dopamine agonists are discussed.

Dopamine metabolism | REM sleep deprivation | Corpus striatum | Frontal cortex | ^{3}H-Spiperone binding | Tyrosine hydroxylase | Dopamine autoreceptor

A NUMBER of investigators have reported pronounced behavioral supersensitivity to directly- and indirectly-acting dopamine (DA) agonists following prolonged rapid eye movement (REM) sleep deprivation. Originally, increases in sexual and aggressive behaviors were observed in the REM sleep-deprived rat following the administration of d-amphetamine [8]. These findings were extended to other DA agonists [22, 23, 24], and have led to the hypothesis that the modification in sensitivity to dopaminergic drugs by REM sleep deprivation is due to a functional hyperactivity of the DA system. Thus, REM sleep deprivation may produce, at least, these possible effects: (1) a change in DA release, (2) a change in DA receptors (pre- or post-synaptic), or (3) a combination of both (1) and (2). The first possibility has gained support from the findings that brains of REM sleep-deprived rats show elevations of tyrosine hydroxylase in brain stem and cortex [20] and of DA levels in striatum [9]. DOPAC levels are elevated six hours into the recovery from REM sleep deprivation [27], but not immediately following REM sleep deprivation [19]. The behavioral changes resulting from the combination of REM sleep deprivation and DA agonist administration also have been explained in terms of either post-synaptic receptor supersensitivity [24] or of pre-synaptic receptor subsensitivity [19]. Both conditions are assumed direct results of REM sleep deprivation.

In order to elucidate more fully the effect of REM sleep deprivation on the DA system we measured both DA turnover and receptor changes following this manipulation. Previous observations have been limited to the nigrostriatal system. Since the mesocortical DA system exhibits unique responses to neuroleptic drugs [2] and stressful situations [7,21], we also included this DA system in our analyses.

METHOD

Male Sprague-Dawley rats (Hotlzman, 250–300 g) were maintained on standard laboratory chow and in a room with a 12 hr light/dark cycle for at least one week before the start of the experiment. The experimental animals (Rd group) were confined for 96 hrs (at 10 a.m.) in individual enclosures, designed to selectively deprive the rats of REM sleep. These enclosures were plastic cages containing a platform 6.7 cm in diameter, surrounded by water (7 cm deep) to within 0.5 cm of the top of the platform. The water in the cages was changed and the animals were weighed daily. Food was available ad lib. This method and weight/platform diameter ratio has been shown effective for 96 hr paradigms in selectively preventing the occurrence of REM sleep but allowing substantial amounts of slow-wave sleep [6, 13, 14, 18]. A second group of rats comprised the stress-control group (Sc).

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These rats were placed in enclosures identical to the Rd group except that the size of the platform was 12.8 cm in diameter. Whereas this condition exposes the animal to a stressful situation comparable to that of group Rd, it allows for the occurrence of REM sleep since the platform is large enough to accomodate a rat in REM sleep postural atonia. A third group of normal control rats were kept in their home cages under the same light/dark conditions as the Rd and Sc groups. All subjects were weight matched and randomly assigned to the three groups.

BIOCHEMICAL ASSAYS

The rats were kept in these conditions for 96 hr and then immediately killed by chloroform asphyxiation. The brains were rapidly removed, chilled in cold saline, the olfactory tubercles removed and the brain cut [10]. After removing the corpus striatum, the tissue rostral to the cut through the anterior commissure was taken as the frontal cortex. The striatum, both anterior and posterior to the anterior cut, was carefully removed so as not to contaminate the frontal cortex sample. Striatal and frontal cortex concentrations of dihydroxyphenylacetic acid (DOPAC) and were determined by organic extraction and fluorophor development [15]. Spiperone binding to striatal membranes was used to determine neuroleptic receptor (D_2) B_{max} and K_d [5]. Briefly, the corpus striatum from each rat was homogenized in 100 vol of 0.05 M Na/KPO_4 buffer, pH 7.4, for 30 sec with a Polytron homogenizer. The membranes were pelleted by centrifugation (18,000 rpm), resuspended in buffer with the Polytron homogenizer, repelleted and resuspended in 100 vol and 0.2 ml aliquots were used for the binding assay (final volume, 2.0 ml). ^{3}H-spiperone (0.05 nM final concentrations, 6000 cpm) was added to each tube and the spiperone concentration ranged from 0.05 nM to 2.0 nM. A concentration of 0.2 μM (+)butaclamol was used to determine non-specific binding. The incubation proceeded for 30 min at 37° and then was stopped by vacuum filtration through Whatman GF/c filters. The tubes were then rinsed twice with 3.0 ml of cold buffer. The specific binding at each triplicate concentration of spiperone was used for a Scatchard analysis to determine B_{max} and K_d. Because of the small total number of receptors in the frontal cortex, including a significant component of $5HT_2$ receptors binding of spiperone, each frontal cortex sample was homogenized in 50 vol, with 0.5 ml of the final membrane suspension used for the assay (incubation volume was still 2.0 ml). The amount of binding to $5HT_2$ and D_2 receptors was determined by adding ^{3}H-spiperone (0.1 nM final concentration, 12,000 cpm) to each tube and then displacing the spiperone with either 0.1 μM mianserine (a $5HT_2$ antagonist with poor affinity for DA receptors) or 0.1 μM mianserine and 0.2 μM (+)butaclamol. The difference between ^{3}H-spiperone trapped on the filters alone and in the presence of mianserine was taken as $5HT_2$ binding, and the binding in the presence of mianserine minus that in the presence of mianserine plus (+)butaclamol was taken as D_2 binding. The concentration of ^{3}H-spiperone was chosen close to its K_d so that the assays would be sensitive to changes in either K_d or B_{max}.

In order to determine whether REM sleep deprivation-induced pre-synaptic DA autoreceptor subsensitivity, synaptosomal tyrosine hydroxylase activity was determined [16] in eight Rd rats and compared to that of normal controls. The 1,000 × g supernatant fluid from striatal homogenates (in 10 vol 0.32 M sucrose) was used as the source of synaptosomes.

TABLE 1

EFFECT OF REM SLEEP DEPRIVATION ON STRIATAL AND FRONTAL CORTICAL DOPAMINE METABOLISM

Condition	DOPACμg/g ±s.e.m. (n) Striatum	Frontal Cx
Normal	1.40 ± 0.15 (6)	0.19 ± 0.02 (6)
Stress-control	1.77 ± 0.12 (8)	0.20 ± 0.01 (8)
REM sleep deprived	2.06 ± 0.11 (6)*	0.25 ± 0.03 (6)

$F(2,17)=5.943$; *$p<0.01$, Dunnett's t-test.

Rats were placed on large (stress control) or small (REM sleep deprived) islands for 96 hrs and killed immediately at the end of isolation.

Dowex 50 purified 3,5-^{3}H-tyrosine (New England Nuclear) was used as substrate (200,000 cpm, 8×10^{-5} M) and the ^3H-H_2O formed in 30 min at 37° used as product. The ^3H-H_2O was separated from tyrosine, L-DOPA and amines by passing the acidified incubation medium through Dowex 50 columns. The eluant was mixed with 13 ml Beckman Readisolv HP and counted in a Beckman LS 200 with external standardization.

Drugs and Sources. Spiperone (Janssen Pharmaceutica, Beerse), mianserine-HCl (Organon International, Oss), (+)butaclamol (Ayerst Research Laboratories, Montreal) and apomorphine-HCl (Sigma Chemical Co., St. Louis, MO).

RESULTS

Both the Rd and Sc groups lost comparable amounts of weight (2–4%) during the experiment. The normal control group gained about 5% of its initial weight over the 4-day period.

Effects of REM Sleep Deprivation on DA Metabolism

Table 1 shows the concentrations of DOPAC obtained from the three groups of rats after 96 hrs of experimental procedure. Four days of REM sleep deprivation (n=6) induced a highly significant elevation in striatal DOPAC relative to normal controls (n=6). Although the DOPAC levels in the Rd group were higher than in the Sc group, these differences did not reach statistical significance. On the other hand, DOPAC levels in the Sc group (n=8) were not significantly higher than those of the normal group. No significant changes in frontal cortex DOPAC were observed in either the Rd (n=6) or Sc (n=8) groups. Thus, the altered DA metabolism was selective for the striatum.

Effects of REM Sleep Deprivation on DA Binding to Post-Synaptic Membrane

As shown in Table 2 no significant changes in spiperone binding (D_2 dopamine receptor) occurred in the striatum or in frontal cortex in either the Rd or Sc groups when compared to normal controls. Both spiperone B_{max} and K_d on striatal membranes were similar in all three groups. Mianserine-displaceable binding ($5HT_2$ serotonin receptor) in frontal cortex was not affected by either the Rd or Sc manipulation.

TABLE 2

EFFECT OF REM SLEEP DEPRIVATION ON STRIATAL AND FRONTAL CORTICAL ^{3}H-SPIPERONE BINDING

	DA_2dopamine receptor binding			$5HT_2$ binding
	Striatum		Frontal Cx	Frontal Cx
Condition	B_{max}	K_d (nM)	bound	bound
Normal	260 ± 17 (6)	0.19 ± 0.01 (6)	7.79 ± 1.00 (10)	16.12 ± 0.90 (10)
Stress-controls	251 ± 12 (6)	0.16 ± 0.01 (6)	7.60 ± 0.63 (6)	15.00 ± 0.92 (6)
REM sleep deprived	260 ± 27 (4)	0.17 ± 0.01 (4)	8.88 ± 1.24 (6)	15.45 ± 1.02 (6)

Measures except K_d, in f Moles/mg protein ±s.e.m. (n).

Rats were placed on large (stress control) or small (REM sleep deprived) islands for 96 hrs and killed immediately at the end of isolation. The B_{max} and K_d for ^{3}H-spiperone binding were determined by Scatchard analysis. Mianserin-displaceable binding was used to determine $5HT_2$ binding in frontal cortex and the difference between binding in the presence of mianserin or mianserin plus (+)butaclamol used to determine D_2 binding.

Tyrosine Hydroxylase Activity as a Measure of Autoreceptor Sensitivity

The measures of inhibition of synaptosomal tyrosine hydroxylase activity by 0.5 μM apomorphine revealed no differences between the Rd and normal control groups (see Table 3). The results indicated no change in autoreceptor sensitivity due to REM sleep deprivation. Furthermore, basal tyrosine hydroxylase activity was the same in both groups.

TABLE 3

EFFECTS OF REM SLEEP DEPRIVATION ON SYNAPTOSOMAL TYROSINE HYDROXYLASE ACTIVITY

	3H-H_2O p Moles/mg/hr ±s.e.m. (n)		
	Control	0.5 μm Apomorphine	% change
Normal	7.96 ± 0.32 (8)	3.80 ± 0.15	−52.3 ± 1.3
REM sleep deprived	7.60 ± 0.21 (4)	3.36 ± 0.33	−55.8 ± 3.4

Rats were placed on small islands for 96 hrs. Striatal synaptosomes were incubated with 3,5-^{3}H-tyrosine and ^{3}H-H_2O used as product to determine the rate of tyrosine hydroxylation. Autoreceptor sensitivity was tested by adding 5×10^{-7} M apomorphine.

DISCUSSION

Four days of REM sleep deprivation induced a highly significant elevation in striatal DOPAC concentration relative to that of normal controls. The stress control group (large platforms) exhibited no significant change in striatal DOPAC concentration. However, there was no significant difference between the DOPAC concentrations in the Rd striata compared to the Sc group, since the Sc DOPAC concentration was between the normal and Rd concentration. This result may be due to an additive effect of isolation stress or motor demand, to REM sleep deprivation, as the platform size is decreased, rather than a selective effect of REM sleep deprivation. Since there was no alteration of striatal synaptosomal tyrosine hydroxylase activity or autoreceptor sensitivity to apomorphine, the increased DOPAC concentration may reflect an increase in either DA nigral impulse flow or incidence of spontaneously active DA neurons.

Unlike footshock stress [7,21] REM sleep deprivation in this study was without effect on frontal cortex DOPAC levels. Indeed the effects of footshock stress vs. REM sleep deprivation appear to be inversely related; footshock stress elevates mesocortical and mesolimbic DOPAC with no effect on striatal DOPAC, whereas REM sleep deprivation elevates striatal DOPAC concentrations with no effect on the mesocortical terminal system. A short period of locomotion in a slowly revolving wheel has an effect similar to that of REM sleep deprivation on striatal DOPAC levels, but is likewise without effect on frontal cortex DOPAC levels (Miller *et al.*, manuscript in preparation).

Numerous investigators have observed behavioral supersensitivity to directly- and indirectly-acting DA agonists following REM deprivation [3, 4, 8, 22, 23, 24]. It has been suggested that such behavioral supersensitivity might represent post-synaptic DA receptor supersensitivity [22]. However, no evidence of change in post-synaptic receptor number or affinity was found for the D_2 receptor in the striatum or for the D_2 or serotonin ($5HT_2$) receptors in the frontal cortex. It must be noted, though, that no assay of the adenylate cyclase linked receptor (D_1) was performed in this study. Behavioral subsensitivity of the putative striatal autoreceptor (reduction in hypomotility induced by small doses of apomorphine) following REM deprivation has been demonstrated [19]. These authors found decreased behavioral response to "pre-synaptic" doses of apomorphine following REM sleep deprivation. Yet, neither their biochemical data nor the data reported here provide any support for DA autoreceptor subsensitivity to DA agonists following REM sleep deprivation. The one report of an elevation in tyrosine hydroxylase activity following REM sleep deprivation [20] may have reflected the activity of norepinephrine-associated tyrosine hydroxylase rather than DA-associated tyrosine hydroxylase because non-dopamine innervated

brain areas were assayed. Our data show that the elevation of DOPAC levels is not due to altered pre- or post-synaptic receptor sensitivity. A more likely hypothesis to explain increased response to DA mimetics is that the increase in DA transmission induced by REM sleep deprivation (as evidenced by the DOPAC changes reported here) is additive to the effects of DA agonists at post-synaptic receptors in the striatum. It is known that REM sleep deprivation has a therapeutic efficacy in the treatment of endogenous depression [25]. In light of our hypothesis, perhaps joint administration of a DA agonist (e.g. bromocriptine) with REM sleep deprivation would have an additive antidepressant effect.

An alternative hypothesis to explain the reports of increased responsiveness to DA mimetics is that alterations in other transmitter systems, distal to the nigrostriatal system, have taken place in the Rd rats. Evidence for such indirect effects has been provided by reports that serotonergic and cholinergic drugs can alter amphetamine-induced stereotyped behavior [11,26]. Furthermore, it is known that sleep deprivation or stress will alter noradrenergic, serotonergic [27] and peptidergic transmission [1,12]. Similarly, it is possible that cholinergic or gabaergic neurons in the nigrostriatal system may be likewise affected by REM sleep deprivation. A small change in DA function, produced by either the increased DA metabolism (Table 1) or alterations in other neuronal systems, can have marked effects on responsiveness to stimulant drugs. For example, the response to apomorphine increases 50% after withdrawal from chronic haloperidol treatment even though DA-receptor binding increases only 25% [17]. However, no changes occurred in ^{3}H-spiperone binding following REM sleep deprivation, which indicates that enhanced behavioral responses to stimulants are not due to altered DA receptor sensitivity. Therefore, either the increased striatal DA metabolism or alterations in the function of other systems, efferent to the striatal DA receptor, are the likely cause of increased sensitivity to stimulant drugs following REM sleep deprivation.

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