Pharmacology Biochemistry & Behavior, Vol. 25, pp. 223–225, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

BRIEF COMMUNICATION

REM Sleep Deprivation Induces a Decrease in Norepinephrine-Stimulated ^{3}H-Cyclic AMP Accumulation in Slices From Rat Brain[1,2,3]

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Received 27 December 1985

TRONCONE, L. R. P., S. BRAZ, M. A. C. BENEDITO AND S. TUFIK. *REM sleep deprivation induces a decrease in norepinephrine-stimulated ^{3}H-cyclic AMP accumulation in slices from rat brain.* PHARMACOL BIOCHEM BEHAV 25(1) 223–225, 1986.—Beta adrenergic sites in rat brain are reduced after repeated treatment with antidepressant drugs, with REM sleep deprivation (REMd) having the same effect. This paper reports the effects of REMSd in the production of ^{3}H-cyclic AMP in frontal cortical slices by NE challenge. Data presented in this paper report a marked decrease in ^{3}H-cyclic AMP synthesis after REMSd, which is in accordance with previous results showing adrenergic receptor down-regulation following REMSd. Results are discussed in view of possible interaction with dopaminergic systems and depression management.

REM sleep Norepinephrine Adenylate cyclase Cyclic AMP

ENHANCED norepinephrine (NE) transmission is characteristic of antidepressant treatments, including drugs and ECT. The delayed onset of the therapeutic effect of these treatments is correlated with the delayed onset of neurochemical alterations such as reduction in beta-adrenergic receptor number and sensitivity of the NE-stimulated adenylate-cyclase [15]. These neurochemical effects are induced by antidepressant drugs such as MAOIs and amine uptake inhibitors, electroconvulsive therapy as well as by REM sleep deprivation (REMSd) [13,17]. As pointed out in a recent review, there is a good correlation between beta-adrenergic receptor down regulation (observed in binding studies) and desensitization of NE-receptor coupled adenylate-cyclase (assayed by cyclic AMP accumulation in brain tissue) during prolonged antidepressant administration [15]. This review also mentioned the need for data concerning NE-receptor desensitization produced by REM sleep deprivation.

REMSd induces alterations of several responses to dopaminergic drugs in animals such as aggressiveness and stereotypy, hypothermia and sexual behavior [3]. These alterations suggest the hypothesis of a dopaminergic supersensitivity produced by this manipulation [16]. Moreover, aggressive behavior induced in REMSd animals can be obtained either by apomorphine or NE-synthesis inhibitors such as diethyl-dithio-carbamate (DDTC) or FLA-63 [2], suggesting a role for NE in the genesis of this behavior. This interaction between NE and dopamine (DA) seems evident in several behavioral tasks such as stereotypy, self stimulation, eating and aggression [1].

As shown earlier [6,9], REMSd induces a down regulation of beta-adrenergic receptors. This alteration is in the same direction as the impairment of NE transmission produced by synthesis inhibitors, suggesting that this phenomenon is involved in the behavior observed in REMSd animals.

Using the synthesis of ^{3}H-cyclic AMP as an index of receptor status, we assayed the NE-stimulated adenylate-cyclase in the frontal cortex of normal and REMSd rats.

[1]Fellowship from Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP).
[2]Fellowship from Conselho Nacional de Pesquisa (CNPq).
[3]This work was partially supported by Associacao Fundo de Incentivo a Psicofarmacologia (AFIP).
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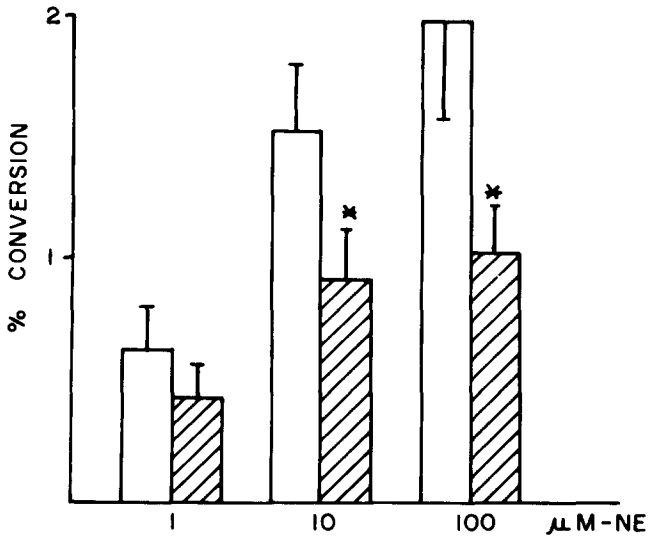

FIG. 1. Percent conversion of ^{3}H-ATP to ^{3}H-cyclic AMP for three norepinephrine concentrations. Open bar=control samples; hatched bar=REM sleep deprived samples. Baseline values were 0.52±0.06 percent converted to cAMP for controls and 0.62±0.2 percent converted for REMSd. Bars represent the mean of three separate experiments, each of which was performed in triplicate. *=$p<=0.05$, Student's t-test. Lines over bars indicate Standard Error of the Mean.

METHOD

Animals

Male adult Wistar rats, weighing from 250 to 350 grams, from our own inbred were used. They were kept in wire cages containing three animals each, with free access to water and food during all procedures, and a light dark cycle of 12/12 hours.

REM Sleep Deprivation

REMSd was effected as described previously [7], by the method of the flower pot and water tank. The REM deprivation lasted 96 hours.

Assay of the NE-Sensitive Adenylate Cyclase

Adenylate cyclase was assayed by the method of prelabeling of the internal pool of ATP with ^{3}H-adenine, as described [11]. Briefly, chopped tissue from 4 to 5 animals was pooled and rinsed with Krebs-Ringer-bicarbonate buffer (pH=7.3), with glucose and incubated for 15 minutes at 37°C under constant O_2-CO_2 (95–5 percent). The slices were transferred to fresh buffer containing 100 μCi of ^{3}H-adenine (final concentration 65 μM) and 30 μM unlabeled adenine and incubated for a further 40 minutes in the same conditions. The slices were filtered in a polyester mesh and washed twice with buffer and then incubated for 15 minutes. Slices were filtered again and 10 to 20 mg wet weight portions were distributed to test beakers containing fresh buffer. After 5 minutes NE was added to a final concentration desired and stimulation lasted 10 minutes. The reaction was terminated by exposing the samples to 5 percent trichloroacetic acid and homogenization by ultrasound, followed by centrifugation at 1700 g for 15 minutes at 4°C. Radioactivity was monitored in a portion of 50 μl of the acid supernatant. Recovery was estimated by adding a known amount of unlabeled cAMP to each sample and inspected spectrophotometrically and ranged from 60 to 90 percent. Radioactive cAMP was assayed by the double column method described elsewhere [10]. Results are presented as the percentage of the total tritium present as ^{3}H-cAMP less basal (percent conversion over basal), corrected for recovery.

Drugs

2-^{3}H-adenine (15.5 Ci/mmol) and Aquasol® were purchased from New England Nuclear. L-arterenol and adenine HCl were purchased from Sigma Chemical Co. All other reagents were of analytical grade.

Statistical analysis employed Student's t-test and $p<0.05$ was considered statistically significant.

RESULTS

Results are presented in Fig. 1 and show that the slices from REMSd animals responded less to stimulation by NE than did controls in two concentrations used ($p<0.05$). The synthesis of ^{3}H-cAMP in slices from REMSd animals was 59 percent of controls for a 10 μM NE concentration and 51 percent for a 100 μM NE concentration. Baseline values were 0.52±0.06 (mean ± SEM) percent converted for controls and 0.62±0.2 percent converted for REMSd animals (not statistically different).

DISCUSSION

The data show that NE-stimulated adenylate-cyclase in the frontal cortex of REMSd rats is less sensitive to NE stimulation. This result is substantiated by data from binding studies [6,9].

The observation that aggressive behavior in REMSd animals induced by administering apomorphine [16] or dopamine-beta-hydroxylase inhibitors [2], favors the hypothesis of an interaction between the NE and DA systems to induce this behavior. Such an interaction is widely described for several different behaviors. In most of them, DA exerts a stimulatory or switching role, while NE appears to exert a modulatory role [8]. As shown in other studies, the dopaminergic system may be supersensitive in REMSd animals thereby facilitating the onset of an aggressive state [16]. The data presented in this study indicate that the possible modulatory effect of the noradrenergic system on this behavior may be down regulated. The summation of these two situations in REMSd rats could be responsible for the aggressiveness observed.

Down regulation of beta-adrenergic receptors is considered to be a desirable effect when treating depression [4]. This effect has been used to explain the therapeutic outcome of antidepressant therapy [4,13]. In view of the results from binding studies and those reported herein, REM sleep deprivation could constitute an alternative form of treatment for affective illnesses.

As reported earlier [5,12], several stressors are also able to induce down regulation and subsensitivity of beta-adrenergic receptors in brain. It is noteworthy that these stress-induced alterations of brain adrenergic receptors range from 10 to 25 percent of the normal levels. In the case of tricyclic antidepressants, the subsensitivity reaches 45 to 50 percent of controls, which could signify a more dramatic change in adrenergic transmission. In REMSd rats the re-

duction observed reached 48 percent, resembling that described for repeated antidepressant drug treatment. In this way, enhanced NE transmission is likely to occur during REMSd which could be responsible for the compensatory subsensitivity observed. This alteration cannot be attributed solely to the stress factor involved in the REMSd procedure as indicated by the range of the induced alteration. Perhaps specific mechanisms are involved.

ACKNOWLEDGEMENTS

We are grateful to Claudia I. P. Macaubas and Julia M. C. Andrade for expert technical assistance.

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