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Enhancement of Tyrosine Hydroxylase Activity by Oxotremorine Does Not Affect Sleep in the Rat[1,2]

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RAMM, P. AND H. TAUB. *Enhancement of tyrosine hydroxylase activity by Oxotremorine does not affect REM sleep in the rat.* PHARMAC. BIOCHEM. BEHAV. 12(3) 353–357, 1980.—Marked enhancement of tyrosine hydroxylase (TH) activity was observed in the rostral pons of Wistar albino rats, 3 days after a single injection of Oxotremorine. TH activity in other brain areas, and regional levels of norepinephrine, dopamine, serotonin and 5-hydroxyindoleacetic acid were unaffected. Enhancement of TH activity did not affect length or number of REM episodes, or the amount of REM occurring in 24 hr. REM occurrence did not, thus, vary in accordance with activity of the rate-limiting enzyme for catecholamine synthesis. These data suggest that, although REM may reflect neuronal protein synthesis, REM is not part of a mechanism regulating the activity of enzymes in the pontine CA synthesis pathway.

Sleep   Oxotremorine   Tyrosine hydroxylase   Catecholamines

THE appearance of rapid eye movement (REM) sleep may be correlated with occurrence of many forms of brain protein synthesis [1, 30, 42]. The hypothesis is supported by reports that: (a) REM is selectively affected by agents which interfere with protein synthesis [9, 12, 21, 31, 33, 37, 43]; (b) protein concentrations in brain perfusates vary cyclically, and are higher during REM than during wakefulness (W) [10]; (c) REM is enhanced by administration of anabolic growth hormone [11,42]. It has been suggested that protein synthesis is necessary for normal function of the mechanism initiating REM [12].

In addition to the view that protein synthesis is associated with activity of a REM-initiating mechanism, is a hypothesis that REM initiation is functional in maintenance of brain protein synthesis. In particular, the catecholamine (CA) maintenance hypothesis [13,41] grew out of evidence that restorative processes are maximal during sleep (see [1] for review), and observations of increased REM following CA-depleting treatments and decreased REM following administration of CA-potentiating treatments (see [41] for review). Stern and Morgane [41] suggest that CA-potentiating agents, by increasing the synaptic availability of catecholamines, fulfill the function of REM. Conversely, CA depletion increases REM need. It is suggested that REM maintains CA homeostasis at the synapse. There is, thus, a hypothesis that REM is linked to neuronal protein synthesis, and a more specific hypothesis suggesting that REM actively participates in regulation of CA synthesis. The latter hypothesis implies that a REM-generation system could sense CA availability and, when necessary, enhance the activity of the enzymes (proteins) participating in CA synthesis.

A possible mechanism for enhancement of CA-synthetic enzyme activity has recently become evident. Acetylcholinesterase (AchE) and choline acetyltransferase are found in the pontine tegmentum [5,24], the point of origin of most brain CA neurons [26]. Further, AchE is evident in presynaptic terminations upon cells in the locus coeruleus (LC) [15]. In light of the neurochemical heterogeneity of the pontine tegmentum, it is of interest that in sympathetic ganglia and adrenal medulla in the periphery [44,46], and in the LC [23], increased activity in presynaptic cholinergic neurons is followed by enhanced postsynaptic activity of the enzyme tyrosine hydroxylase (TH). TH, a protein, is rate-limiting for CA synthesis [38]. As cholinergic neurons are implicated in the generation of REM (see [19] for review), transynaptic regulation of TH is a means by which cholinergic activity could influence both REM and CA synthesis. The present study was conducted to examine the effects of transynaptic

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enhancement of TH activity upon REM. If REM regulates activity of enzymes in the CA synthesis pathway, enhancement of activity of the rate-limiting enzyme should lead to decreased REM need and decreased REM time.

A single administration of the muscarinic cholinergic agonist, 1-[4-(1-Pyrrolidinyl)-2-butynyl]-2-pyrrolidinone (Oxotremorine), results in cholinergically-mediated, prolonged (lasting more than one week post-injection) activation of TH in the LC [23]. The LC gives rise to major noradrenergic projections (see [2] for review), and has been implicated in the occurrence of the REM state [18,41]. Oxotremorine was administered to rats and EMG and EEG recordings were made during the following fifteen days. The 15 day recording period encompassed the time of previously reported TH activation and subsequent return to baseline levels [23]. Other rats received Oxotremorine, and assays of TH activity and levels of the biogenic amines were conducted at 3 days post-administration. The assays occurred at the time during which TH activity has been previously reported to be maximal [23]

METHOD

Surgery and Recording Parameters

Seven 450–600 g, 4–6 month old, male Wistar albino rats (CBFL) were anesthetized with sodium pentobarbital (55 mg/kg) and were implanted with chronic electrodes for the recording of fronto-occipital EEG and neck EMG. The animals were allowed 2–3 weeks of postoperative recovery in individual $10\times25\times18$ cm rack cages. They were then placed in individual $23\times30\times18$ cm recording cages situated in a quiet room and connected, via flexible cables, to mercury pool commutators which permitted free movement. A 12 hr on, 12 hr off light-dark cycle was maintained with the lights on at 10:00 hr and off at 22:00 hr. Food and water were available ad lib. Animals were allowed one week to acclimatize to the recording situation. Continuous recordings were then taken on a Beckman type RM Dynagraph, at a chart speed of 2.5 mm/sec. Light period recording was initiated at 10:30 hr and terminated at 21:30 hr. Dark period recording was initiated at 22:30 hr and terminated at 09:30 hr. The 1 hr period between recording sessions was used for routine maintenance tasks. Polygraph records were obtained for four baseline days. At 10:00 hr of the fifth light period, each animal was injected subcutaneously with 1.5 mg/kg of Oxotremorine (Aldrich) in 0.5 ml of isotonic saline. Recordings were made during the day of injection, and subsequently on Days 2, 3, 4, 7, 9, 12 and 15 following injection.

Scoring of Data

Scoring of data involved simultaneous evaluation of EEG and EMG records in successive 30 sec epochs. Sleep-wake activity during the light, dark, and total (light+dark) recording periods fell into four categories: wake (W), light sleep (SWS1), deep sleep (SWS2) and REM, as described by Lidbrink [25]. SWS1 and SWS2 were also combined to give a measure of total SWS. It was not known, during scoring, whether the data under consideration was from the baseline or post-drug periods. Reliability was assessed by the method of Chichetti and Allison [6] who suggest that reliability coefficients of 0.96 or above are acceptable. In this method, scoring reliability is expressed as a proportion of agreement over repeated independent scorings of data blocks. The reliability coefficient obtained in the present case was 0.98.

Neurochemistry

Thirty-two male 300–600 g, 3–6 month old, Wistar albino rats (CBFL) received the surgical and housing treatments described above, with the single exception that during the recovery period they were housed in rack cages. Oxotremorine was injected, as above, into sixteen rats. Sixteen rats received 0.5 ml of isotonic saline. Seventy-four to 77 hr following injection, alternate animals from each treatment condition were removed and sacrificed by decapitation. The brains were rapidly removed and dissected into the rostral pons, midbrain, motor cortex, hippocampus and corpus striatum. After weighing, samples were frozen in liquid nitrogen and stored at −75°C until the following day. TH activity was assayed by the method of McGeer *et al* [28] as modified by Peters and Tang [32]. This method involves the conversion of L-(C^{14}) tyrosine to L-(C^{14}) dopa using brain homogenates subjected to a solubilization procedure. The solubilized tyrosine hydroxylase was assayed in the presence of added synthetic pteridine co-factor. The L-(C^{14}) dopa produced was isolated on alumina columns and the radioactivity measured in a Nuclear Chicago Mark I liquid scintillation system. Enzyme activity is expressed as nM dopa formed per hr per g wet weight, with a small correction made for endogenous tyrosine levels.

NE and dopamine (DA) were assayed spectrofluorometrically by the method of Laverty and Taylor [22], after extraction from an acidified butanol homogenate [27]. Levels of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were determined by the method of Curzon and Green [8]

RESULTS

Neurochemistry

The effects of 1.5 mg/kg of subcutaneously injected Oxotremorine are shown in Table 1, for a time approximately 76 hr after injection. TH activity was markedly elevated in the rostral pons ($p<0.01$, Scheffe), a region containing the locus coeruleus. TH activity was not, however, altered significantly in any of the other brain regions studied. Levels of NE, DA, 5-HT and 5-HIAA were not altered in any brain region ($p>0.05$, Scheffe) (Table 2).

Acute Effects of Oxotremorine

The tremorogenic effect of Oxotremorine was observable within 5 min of injection. Tremor lasted approximately 4 hr, was maximal in the first half hour, and was accompanied by enhanced EMG and desynchronized EEG activity.

Sleep-wake activity during the light, dark and total recording periods immediately following Oxotremorine administration was compared to baseline data using the statistical method of Scheffe [47]. During the light period, W was elevated while SWS1, SWS2 and REM were all below baseline levels ($p<0.05$). During the dark period, W fell below baseline ($p<0.05$), SWS1 was unaffected ($p>0.05$) and SWS2 and REM were above baseline levels ($p<0.05$). Over the total recording period, only W was significantly affected, being elevated above baseline levels ($p<0.05$). Rebound of the sleep states thus occurred, and the animals recovered from drug-induced sleep loss within 24 hr of drug administration.

TABLE 1

THE EFFECT OF ACUTE OXOTREMORINE TREATMENT ON THE TYROSINE HYDROXYLASE ACTIVITY IN RAT BRAIN REGIONS

Brain region	Control (nM DOPA/g/hr)	N	Oxotremorine	N	% Control
Rostral pons	47.11 ± 1.77	8	64.39 ± 1.29	8	137*
Midbrain	50.57 ± 2.64	6	46.37 ± 1.84	8	92
Motor cortex	11.19 ± 0.72	8	10.81 ± 0.60	8	97
Hippocampus	16.07 ± 1.17	8	16.79 ± 0.91	8	104
Striatum	220.80 ± 5.66	8	209.41 ± 8.22	8	95

A single dose of oxotremorine (1.5 mg/kg) was injected subcutaneously. Rats were killed at 74–77 hr postinjection, and total tyrosine hydroxylase activity assayed in the various brain regions (± SEM).

*$p<0.01$ (Scheffe).

TABLE 2

MONOAMINE LEVELS IN RAT (N=8) BRAIN REGIONS, 74–77 HOURS AFTER A SINGLE INJECTION OF OXOTREMORINE (± SEM)

	5-HT		5-HIAA (ng/g)		NE		DA	
Brain region	Control	Oxotremorine	Control	Oxotremorine	Control	Oxotremorine	Control	Oxotremorine
Hippocampus	654 ± 28	668 ± 18	664 ± 54	720 ± 19	624 ± 39	622 ± 32	—	—
Hypothalamus	1884 ± 207§	1893 ± 231§	1008 ± 88	952 ± 148	1615 ± 81	1548 ± 109	—	—
Midbrain	1139 ± 44	1254 ± 31	1232 ± 60	1332 ± 48	618 ± 21	646 ± 22	693 ± 84†	679 ± 106†
Motor cortex	474 ± 20	491 ± 20	408 ± 19	424 ± 14	298 ± 20	280 ± 12	381 ± 54‡	398 ± 71‡
Rostral pons	689 ± 33	720 ± 65	796 ± 27	896 ± 64	659 ± 30	640 ± 37	—	—
Striatum	1036 ± 38	1046 ± 51	912 ± 38	948 ± 36	628 ± 42	620 ± 39	11410 ± 1522*	13316 ± 3343*

*N=2; †N=3; ‡N=5; §N=6.

Long-term Effects upon Sleep-wake Activity

Lewander *et al.* [23] assayed TH activity on *postdrug days* (PD) 1, 2, 3, 4, 5, 7, and 14. TH activity was significantly elevated above control levels on PD 2, 3, 4, 5, and 7. TH activity was not significantly elevated on PD 14. A set of orthogonal weights was constructed [48], a priori, to compare baseline (B) data from the four days before (B1–B4) and a day subsequent (B5=PD15) to the reported period of TH activation, with data from the period of maximal reported TH activation (PD 3, 4, 5, 7). Postdrug Day 2 was excluded from the comparison to minimize influence of the acute sleep disruption caused by Oxotremorine administration. Sleep-wake activity is presented in Fig. 1 and in Table 3. Analyses of variance revealed no significant variation in sleep-wake time during the light period ($p>0.05$). During the dark recording period, W was decreased, $F(1,6)=7.39$, $p<0.05$, and total SWS enhanced, $F(1,6)=14.79$, $p<0.01$. During the total recording period, no significant variation occurred in sleep-wake time ($p>0.05$).

Further analyses of variance were performed, comparing the length and number of REM episodes during baseline (B3–B4) and PD 3–4 total recording periods. No significant variation was observed in length or in number of REM episodes ($p>0.05$).

TABLE 3

SLEEP-WAKE ACTIVITY (MIN) DURING BASELINE AND POST-DRUG TOTAL RECORDING PERIODS (± SD)

	Baseline	Post-drug
W	444.1 ± 45.1	457.2 ± 50.9
SWS 1	203.1 ± 47.5	202.9 ± 46.5
SWS 2	550.0 ± 50.6	544.5 ± 46.3
Total SWS	758.0 ± 36.8	747.3 ± 48.9
REM	117.7 ± 15.6	114.8 ± 11.3
Length of REM	1.6 ± 0.4	1.6 ± 0.3
Number of REM episodes/22 hr	74.4 ± 15.3	74.9 ± 12.5

DISCUSSION

Neurochemistry

Our results, demonstrating enhancement of TH activity following Oxotremorine, may be viewed as confirmation of an earlier report of TH activation [23]. That is, the TH enhancement observed may reflect activation of TH resulting from increased activity of the enzyme molecules, rather than increased production of enzyme (induction). Our failure to observe changes in monoamine levels and 5-HT turnover suggests that, during the postdrug period, animals experienced a selective enhancement of TH activity. Our data is, however, incomplete with respect to DA, which may have varied from control levels. Our failure to demonstrate enhanced TH activity in terminal projection areas of CA neurons (e.g., hippocampus, motor cortex) may result from dilution of TH as it is transported to diffuse terminal projec-

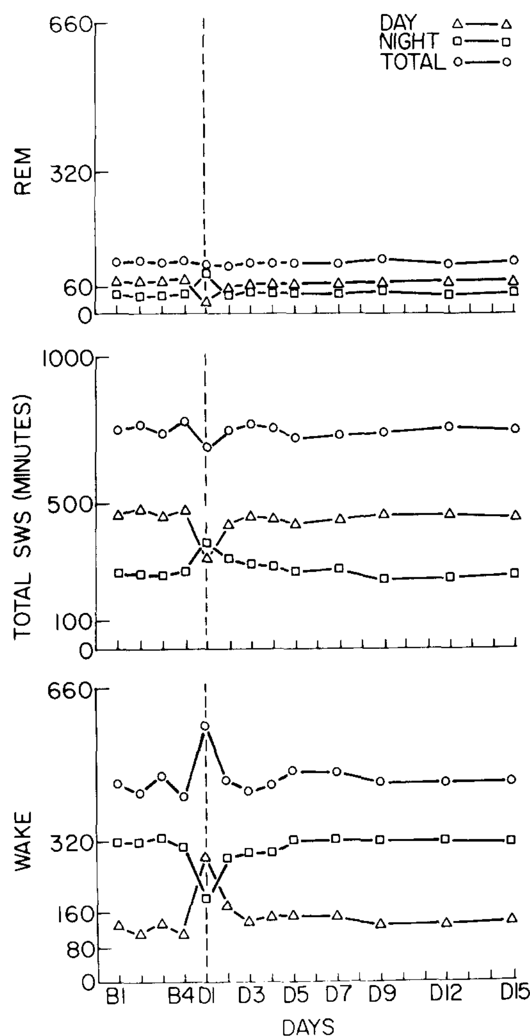

FIG. 1. Sleep-wake activity during baseline (B) and postdrug (D) recording periods. Oxotremorine (1.5 mg/kg) was administered on D1.

tion areas. Such dilution has been suggested to follow TH induction by reserpine [35]. The observed normal TH activity may also have been a function of the time course of TH transport from the cell body (approximately 2 mm/day in rat brain) [3]. After reserpine administration, for example, increased TH activity appears first in adrenergic cell bodies, and several days later in the terminals of sympathetic neurons [45]. Similarly, LC TH activity peaks 3 days after reserpine, but TH activity does not peak until 6–11 days postadministration in cerebellum, 11 days in frontal cortex and 8–11 days in hypothalmus [3, 49, 50]. It is possible that after Oxotremorine administration, also, enhanced TH activity would be evident in other brain regions following transport of affected TH to the projection sites of pontine neurons. If such transport did occur, it did not exert observable effects upon sleep-wake activity during the two weeks following Oxotremorine administration.

REM

There is ample evidence that aminergic activity can influence REM, but the mechanism by which the influence is exerted is unclear. If REM is linked to maintenance of CA availability, the REM-induction system must sense and respond to REM need. The present data suggest that REM induction is not a response to selective changes in TH activity, unaccompanied by variation in amine levels. The influence of aminergic neurons upon REM is not, thus, a function of activity of CA synthesis enzymes in the cell bodies of pontine neurons. Synaptic levels of monoamines, however, modulate REM, and we cannot exclude the possibility that REM is linked to enzyme activity in CA terminals. As we observed enhanced TH activity only in the rostral pons, our failure to find variation in REM may have resulted from localization of enhanced TH activity within pontine perikarya.

It is possible, thus, that REM mediates some aspect of synthesis activity in CA terminals. The hypothesis predicts that manipulation of terminal synthesis activity, and destruction of CA terminals should exert major effects upon REM. There is, however, evidence that these treatments exert only minor effects upon REM (see [34] for review). Briefly, reserpine administration is followed by TH induction [3, 29, 36, 49] and administration of alpha-methyl-para-tyrosine is followed by TH inhibition [38]. Despite the opposite effects of these agents upon TH activity, administration of either is often followed by REM increase [7, 20, 39, 40]. Further, in the cat, REM is only transiently disrupted by lesions in the midbrain or pontine tegmentum [4, 14, 16, 17], which disrupt ascending CA projections. Similarly, REM is not disrupted following 6-hydroxydopamine-induced destruction of noradrenergic terminals in the rat [25].

Although REM may be functional in the processes of neuronal protein synthesis, the state is not consistently affected by destruction of CA neurons, by CA depletion, or in the present case, by enhancement of the activity of the rate-limiting enzyme for CA synthesis. A specific link between REM occurrence and neuronal CA synthesis is, thus, not supported.

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