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BRIEF COMMUNICATION

Effect of Repeated Treatment With Electroconvulsive Shock (ECS) on Serotonin Receptor Density and Turnover in the Rat Cerebral Cortex

GABRIEL NOWAK[1] AND JERZY DULINSKI

Institute of Pharmacology, Polish Academy of Sciences, 31-343 Krakow, Smetna 12, Poland

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NOWAK, G. AND J. DULINSKI. *Effect of repeated treatment with electroconvulsive shock (ECS) on serotonin receptor density and turnover in the rat cerebral cortex.* PHARMACOL BIOCHEM BEHAV **38**(3) 691–694, 1991.—The effect of repeated treatment with electroconvulsive shock (ECS) on 5-HT_{1A} and 5-HT_2 serotonin receptor density and on serotonin receptor turnover was measured in the rat cerebral cortex. Repeated treatment with ECS produced an increase in the density of 5-HT_{1A} and tended to increase the specific binding of 5-HT_2 serotonin receptors. However, the turnover of those receptors remained unchanged. These results are in agreement with previous studies which indicate that repeated ECS treatment up-regulates 5-HT_2 receptors and support the contention that 5-HT_{1A} receptors are up-regulated after repeated treatment with ECS; moreover, these data demonstrate that the dynamic characteristics (turnover rates) of serotonin receptors (5-HT_{1A}, 5-HT_2) are unaffected after repeated ECS administration.

Repeated ECS 5-HT_{1A} 5-HT_2 Receptor binding Receptor turnover

REPEATED treatment with ECS produces many changes in the central serotonergic system. The most consistent effect is an up-regulation of serotonin 5-HT_2 receptors [see (5) for review]. In contrast, experiments examining the effect of repeated ECS treatment on 5-HT_{1A} receptors have yielded equivocal results (5). In order to clarify the effect of repeated treatment with ECS on the central serotonergic system, we measured both the static (equilibrium; B_{max}, K_D) and dynamic (turnover) characteristics of 5-HT_{1A} and 5-HT_2 receptors in rat cerebral cortex.

METHOD

Animal Experiments

Male Wistar rats, weighing 180–220 g at the beginning of treatment, were housed under standard laboratory conditions. ECS (150 mA, 0.5 s, 50 Hz AC) was administered via ear clip electrodes once daily for 10 days. Ear clips were attached to controls but no current was passed. Twenty-four hours after the last treatment, the rats received N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, Aldrich Chemie, 10 mg/kg IP) or vehicle (1% Tween 80) in a volume of 8 ml/kg. Animals were sacrificed immediately or 1, 3, 5 or 7 days after EEDQ administration. The brains were removed to ice-chilled plate. The cerebral cortices (neocortex) were dissected and placed immediately on solid CO_2.

Radioligand Binding Assay

Tissue preparation. The cortex was homogenized in 50 volumes (w/v) of an ice-cold Tris HCl buffer (50 mM, pH 7.7 at 25°C) using a Polytron homogenizer. The homogenates were centrifuged at 25,000 × g for 10 min; pellets were resuspended in the same volume of buffer and the homogenate was again centrifuged at 25,000 × g for 10 min. After the second resuspension in Tris buffer, the homogenates were divided into 2 aliquots. One aliquot was further processed for ^{3}H-8-OH-DPAT assays (incubated at 37°C for 10 min, centrifuged and suspended in 100 volumes of Tris HCl buffer containing 10 μM pargyline, 5.7 mM $CaCl_2$ and 0.1% ascorbic acid) while the other was further diluted to 200

[1]Requests for reprints should be addressed to Dr. Gabriel Nowak, Laboratory of Neuroscience, NIDDK, National Institutes of Health, Bldg. 8, Room 111, Bethesda, MD 20892.

volumes with Tris buffer for ^{3}H-ketanserin binding assays.

5-HT$_{1A}$ receptors (^{3}H-8-OH-DPAT). For single point determinations, ^{3}H-8-OH-DPAT (0.4–0.6 nM, spec. act. 183 Ci/mmol, Amersham) was added to 1 ml aliquots of membrane suspension. Nonspecific binding was defined in the presence of 10 μM serotonin with a final assay volume of 1.2 ml. In saturation experiments, six concentrations (0.125–4.0 nM) of ^{3}H-8-OH-DPAT were used. All samples were incubated for 15 min at 37°C. Bound ligand was separated by rapid filtration over glass fiber filters (Whatman GF/B) and rinsed 3 times with 5 ml of ice-cold buffer.

5-HT$_2$ receptors (^{3}H-ketanserin). ^{3}H-Ketanserin (0.4–0.6 nM, spec. act. 61.0 Ci/mmol, NEN) was added to 1 ml aliquots of membrane suspension. Nonspecific binding was defined in the presence of 1 μM mianserin with a final assay volume of 1.2 ml. The samples were incubated for 30 min at 25°C. Bound ligand was separated by rapid filtration over glass fiber filters (Whatman GF/B) and rinsed 3 times with 5 ml of ice-cold Tris HCl buffer.

The filters were then placed in scintillation minivials with 3 ml of Beckman Ready Value™ liquid scintillation cocktail. Radioactivity was measured in a Beckman LS 3801 liquid scintillation counter with 50% efficiency. All assays were performed in duplicate.

Calculations

The irreversible receptor inactivating method, proposed by Maguer et al. (8) for measuring the receptor turnover in a culture tissue, has also been used by Leff et al. (7) for ex vivo experiments. The latter authors used EEDQ, a highly reactive receptor inactivating agent (2), for in vivo receptor inactivation and measured the receptor recovery by in vitro receptor binding techniques.

The calculation is based on the discovery of receptor synthesis and degradation processes, demonstrated by Devreotes and Fambrough (4) and Reed and Lane (13), according to the equation:

$$\ln \frac{R_{SS}}{R_{SS}-R_t} = kt,$$

where R_{SS} = steady-state (static) specific binding, R_t = specific binding measured at time t.

Such a calculation used for measuring the receptor turnover in a culture tissue is applicable with some reservations for ex vivo experiments [(1,7); see (12) for review]. The degradation rate constant k is the slope of the semilogarithmic plot of the receptor recovery after an irreversible inactivation by EEDQ. The synthesis rate constant r is calculated according to the formula: $r = k R_{SS}$.

The equilibrium values (B_{max} and K_D) were calculated by Scatchard analysis of the saturation isotherms (14).

Statistical Analysis

Overall statistical significance was assessed with analysis of variance (ANOVA). Treatment effects were examined with protected least significant difference (LSD) post hoc determinations. Effects were deemed significant when $p<0.05$.

RESULTS

EEDQ, at a dose 10 mg/kg IP, produced a 73% reduction in the density (B_{max}) of 5-HT$_{1A}$ serotonin receptors in rat cerebral cortex 24 h after EEDQ administration (Table 1), and a 70% reduction in the specific binding of 5-HT$_2$ receptors (data not shown).

TABLE 1

EFFECT OF REPEATED TREATMENT WITH ECS OR SINGLE ADMINISTRATION OF EEDQ ON ^{3}H-8-OH-DPAT BINDING TO 5-HT$_{1A}$ SEROTONIN RECEPTORS IN THE RAT CORTEX

	B_{max}		K_D
	pmol/g	%	nM
Control	2.82 ± 0.180	100	0.70 ± 0.088
ECS	3.80 ± 0.130	135*	0.89 ± 0.118
Control	2.58 ± 0.176	100	0.72 ± 0.233
EEDQ	0.70 ± 0.020	27†	0.88 ± 0.105

The rats were killed 24 h after the last treatment with ECS or EEDQ administration (10 mg/kg IP). The results are expressed as mean ± SEM of 3 Scatchard analyses.

*$p<0.02$, †$p<0.01$

Repeated treatment with ECS increased the density of 5-HT$_{1A}$ receptors (35%, Table 1). Repeated ECS treatment resulted in an increase in the specific binding of 5-HT$_2$ receptors (control 3.70 ± 0.05 pmol/g tissue, ECS 5.07 ± 0.58 pmol/g tissue), although this effect did not reach statistical significance ($p<0.06$). In contrast, repeated ECS treatment did not affect the recovery after EEDQ-induced inactivation of 5-HT$_{1A}$ receptors (inset in Fig. 1), or of 5-HT$_2$ receptors (data not shown). The EEDQ recovery data were transformed to semilogarithmic plots and a graphic example of 5-HT$_{1A}$ receptors is shown in Fig. 1. The effect of repeated treatment with ECS on the turnover parameters of 5-HT$_{1A}$ and 5-HT$_2$ receptors are presented in Table 2. ECS treatment did not significantly affect the slope derived from this transformation, which equals the degradation rate constant, for either 5-HT$_{1A}$ or 5-HT$_2$ receptors.

DISCUSSION

Repeated treatment with ECS results in adaptive changes in the brain, some of which may be related to the mechanism(s) of its antidepressant activity [see (5)]. Unfortunately, many of the adaptive phenomena that have been reported are not easily reconciled and in some cases appear to be flatly contradictory. This is particularly true of the serotonergic system.

Repeated ECS results in an up-regulation of 5-HT$_2$ receptors [(5); present results], while its reported effect on 5-HT$_{1A}$ receptors are not consistent. Newman and Lerer (11) found that ECS treatment resulted in a functional subsensitivity of 5-HT$_{1A}$ receptors (inhibition of forskolin-stimulated adenylate cyclase activity

TABLE 2

EFFECT OF REPEATED TREATMENT WITH ECS ON SEROTONIN RECEPTOR TURNOVER PARAMETERS IN THE RAT CEREBRAL CORTEX

	Control	ECS
	5-HT$_{1A}$ Receptors	
r	0.0071	0.0085
k	0.0055	0.0061
	5-HT$_2$ Receptors	
r	0.0873	0.0908
k	0.0236	0.0264

r values are expressed in pmol/g of tissue/h; k values are expressed in h^{-1}.

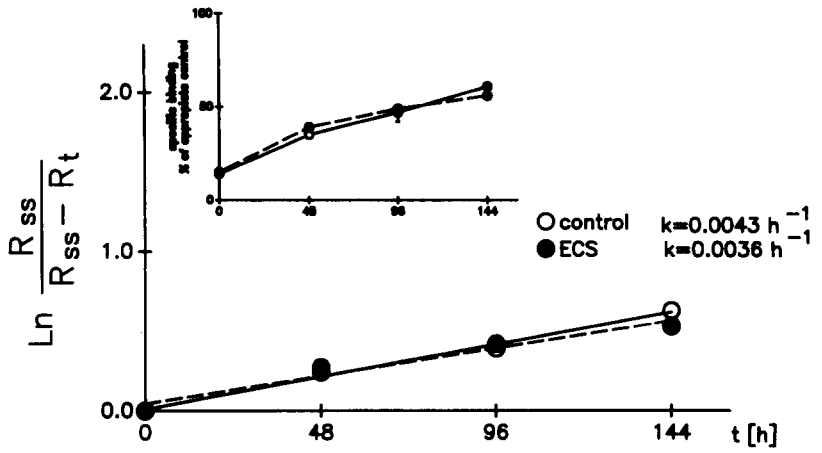

FIG. 1. Semilogarithmic and nontransformed (inset) plots of the time course of the cortical 5-HT_{1A} receptor recovery after EEDQ administration in sham-control rats (○) and those treated repeatedly with ECS (●). The degradation rate constants k are a slope of the appropriate straight lines. R_{SS} = steady-state (static) binding of sham-control or ECS-treated animals. R_t = binding at the time t (the measurements were started 24 h after EEDQ administration). The respective residual specific binding in both sham- and ECS-treated rats (specific binding at 24 h after EEDQ) were subtracted from the recovery and control data points. Each point represents the mean (mean ± SEM, inset) of 4–6 determinations.

by serotonin) which corresponds well with reported behavioral results [see (5)]. In contrast, repeated ECS treatment enhanced locomotor activity induced by 8-OH-DPAT (6) and increased responsiveness to 5-HT (9,10). Moreover, Shapira et al. (15) observed an enhanced prolactin response to fenfluramine in depressed patients successfully treated with ECS, suggesting enhanced responsiveness to 5-HT in these patients. The latter data are in line with our present results from binding experiments.

We have demonstrated that EEDQ produced a reduction in the density (B_{max}) of 5-HT_{1A} serotonin receptors without changing the affinity (K_D). This effect is similar to that obtained previously for 5-HT_2 receptors (1). Using an EEDQ-induced irreversible receptor inactivation method, these authors also demonstrated that 5-HT_2 receptor turnover was reduced in senescent rats compared to young animals, thus explaining the reduction in 5-HT_2 receptor density observed in aged rats. In contrast, no alterations in either 5-HT_{1A} or 5-HT_2 receptor turnover were observed in data presented here. Thus changes in receptor turnover do not appear to account for the changes in 5-HT_{1A} or 5-HT_2 receptor density observed after repeated ECS treatment. It should be noted, however, that this method of measuring receptor turnover required sampling tissue from animals over a period of 7 days after last ECS treatment. Thus changes in receptor turnover could be too rapid to be detected by this method. Reports have appeared during the preparation of this manuscript which suggest that measurement of receptor mRNA could provide relevant data for the period immediately following ECS treatment [e.g., (3)].

Nevertheless, present results are in agreement with previous studies which indicate that repeated treatment with ECS up-regulates cortical 5-HT_2 receptors and support the contention that 5-HT_{1A} receptors are up-regulated either; moreover, present data demonstrate that the dynamic characteristics (turnover rates) of the rat cortical serotonin receptors (5-HT_{1A}, 5-HT_2) are unaffected by repeated ECS administration.

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