Pharmacology Biochemistry & Behavior, Vol. 22, pp. 683–687, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Increased Sensitivity to Serotonergic Agonists After Repeated Electroconvulsive Shock in Rats

MARIAN WIELOSZ

Department of Pharmacology, Medical School, Jaczewskiego, 8, PL-20-090 Lublin, Poland

Received 10 September 1984

WIELOSZ, M. *Increased sensitivity to serotonergic agonists after repeated electroconvulsive shock in rats.* PHARMACOL BIOCHEM BEHAV 22(5) 683–687, 1985.—The effect of single and repeated electroconvulsive shock (ECS) (once daily for 7 days) on head twitches produced by 5-HT agonists (LiCl, 5-hydroxytryptophan; 5-HTP and 5-methoxytryptamine; 5-MT) was investigated 1 hr, 24 hr, 5 days and 10 days after the last ECS, while locomotor activity induced by serotonergic agonists (fenfluramine, 3-chlorophenylpiperazine; m-CPP) and antagonists (metergoline, cyproheptadine) was only investigated after 24 hr. 5HT and 5-HIAA concentrations were measured 0.5, 1 and 24 hr after a single ECS and up to 10 days after repeated ECS. Head twitches induced by LiCl were significantly depressed 1 hr after both single and repeated ECS. The number of head twitches produced by LiCl, 5-HTP or 5-MT given 24 hr after single or repeated ECS did not change but it rose significantly 5 and 10 days after the last shock. Repeated ECS increased locomotor activity 24 hr after the last shock. This increase was significantly enhanced by serotonergic antagonists. Biochemical assays showed that a single ECS did not significantly change brain 5-HT and 5-HIAA concentrations 0.5, 1 or 24 hr after the ECS. On the other hand, repeated ECS raised brain 5-HIAA 0.5, 1 and 24 hr or 5 and 10 days and 5-HT 0.5 hr after the final ECS. It is concluded that a single or repeated ECS both depress the serotonergic system response to LiCl but repeated ECS facilitates the response to serotoninomimetics.

Electroconvulsive treatment Head twitches Locomotor activity 5-Hydroxytryptamine 5-Hydroxyindoleacetic acid

ELECTROCONVULSIVE shock (ECS) is generally thought to be the most effective means of treating endogenous depression [1,8]. Several studies have shown that repeated administration of ECS alters neurotransmitter receptor processes in the rat brain [24, 26, 27, 30]. Repeated ECS reduces the density of β-adrenergic receptors in the rat brain [2, 10, 14] and induces subsensitivity of both central α_2-adrenoreceptors [12,21] and dopamine autoreceptors located within the substantia nigra [3]. Kellar *et al.* [15] and Vetulani *et al.* [25] found that ECS treatment may lead to an increase in the density of 5-HT_2 receptors but did not change the density of 5-HT_1 receptors in the rat frontal cortex. Such an increase in the density of serotonin binding sites may be responsible for functional hypersensitivity of the serotonergic system. Furthermore, a single ECS depresses 5-HT turnover in the rat frontal cortex, an effect which may disappear after prolonged ECS treatment [23]. The mechanism involved in the development of changes is not clear. Therefore, the present study was designed to test the time-course of the action of single and repeated ECS on the central serotonergic system in rats.

METHOD

Animals

Male Wistar rats weighing 160–200 g at the beginning of the study were used. They were kept in colony cages (8 rats) with a natural light-dark cycle, with free access to food and water throughout the experiment. Pharmacological testing was done between 09.00 a.m. and 04.00 p.m.

ECS Treatment

ECS (150 mA, 50 cpm, 0.3 sec) was applied without anaesthesia through ear-clip electrodes. Animals received either a single ECS or one ECS daily for 7 days. Control animals were handled in the same way as shocked rats. Behavioral and biochemical studies were performed 1 hr, 24 hr, 5 and 10 days after delivery of the last shock.

Head-Twitches

Head-twitches were produced by intraperitoneal (IP) injection of 150 mg/kg of 5-hydroxytryptophan (5-HTP) or LiCl to drug-naive rats, or by IP injection of 2.5 mg/kg of 5-methoxytryptamine (5-MT) to rats pretreated IP 30 min earlier with 50 mg/kg of pargyline. Rats were placed separately in Plexiglas cages (25×15×10 cm) and head twitches were counted immediately for 60 min after administration of the serotoninomimetics.

Locomotor Activity

Locomotor activity of individual rats was measured in circular photocell activity cages equipped with 2 light sources and opposite them 2 photoelectric cells 1 cm above a floor.

TABLE 1

EFFECT OF SINGLE AND REPEATED ECS ON HEAD-TWITCHES INDUCED BY 5-HPT, LiCl AND 5-MT

Time After The Last ECS	Number of Head Twitches (Mean ± SEM)		
	5-HTP	LiCl	5-MT
	Single ECS		
Control	49 ± 8.4	21 ± 4.2	33 ± 8.9
1 hr	30 ± 6.9	3 ± 0.6†	38 ± 15.6
24 hr	58 ± 6.6	24 ± 5.6	36 ± 4.8
	Repeated ECS		
Control	42 ± 7.4	28 ± 4.3	28 ± 2.6
1 hr	60 ± 12.6	14 ± 3.8*	25 ± 2.8
24 hr	52 ± 9.9	36 ± 6.3	29 ± 4.2
5 days	96 ± 15.3*	56 ± 9.1*	59 ± 11.1*
10 days	82 ± 14.2*	51 ± 7.2*	55 ± 7.1*

L-5-HTP (150 mg/kg) and LiCl (150 mg/kg) were administered IP 5-MT (2.5 mg/kg) was given IP 30 min after pargyline 50 mg/kg. Head twitches were counted over a period of 60 min after the serotoninomimetics. The control group tested after repeated ECS consisted of 12 rats, all other groups 6. Significantly different from controls.

*$p<0.05$.

†$p<0.001$.

Crossing of the light beams were recorded on a cumulative recorder. The effect of repeated ECS on the locomotor activity of drug-naive control rats and animals treated with serotonergic agents was recorded during 60 min sessions. The serotonergic agents were given IP 30 min before the test at the following doses: fenfluramine 5 and 10 mg/kg, m-CPP 5 mg/kg, metergoline 1 and 5 mg/kg and cyproheptadine 2 mg/kg. Control animals were injected with appropriate vehicle and no difference in locomotor activity was observed.

Biochemistry

For biochemical determinations animals were killed by a guillotine, and their brains were rapidly removed, immediately frozen at −18°C and stored in a freezer for up to 2 days.

The concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were determined in the whole brain spectrofluorometrically according to the method of Curzon and Green [6]. Recoveries for the whole procedure were 82.8% for 5-HT and 93.2% for 5-HIAA.

Drugs

5-methoxytryptamine and L-5-hydroxytryptophan (Sigma, St. Louis) fenfluramine HCl (Servier, Paris, France), 3-chlorophenylpiperazine (CPP, Angelini, Roma, Italy), lithium chloride (Chemapol, Praha, Czechoslovakia) metergoline maleate (Farmitalia Carlo Erba, Milan, Italy), cyproheptadine HCl (Merck, Darmstadt, FRG), pargyline (Germed, Magdeburg, GDR). 5-HTP, 5-MT and metergoline were suspended in 0.5% aqueous carboxymethylcellulose solution. The other drugs were dissolved in distilled water. All drugs were injected IP in a volume of 0.5 ml/100 g body weight. Drug doses were expressed in terms of salts.

Statistics

Statistical significance of the differences was calculated by Student's *t*-test.

RESULTS

Head Twitches

Pretreatment of rats with single or repeated ECS reduced the number of head twitches induced by LiCl given 1 hr after the last shock, but did not change the number of head twitches induced by 5-HTP or 5-MT (Table 1). When 5-HT agonists (5-HTP, 5-MT or LiCl) were injected 24 hr after single or repeated ECS the number of head twitches was almost the same as in control rats. Repeated ECS significantly increased the head twitches induced by LiCl, 5-HTP and 5-MT given 5 or 10 days after the last shock (Table 1).

Locomotor Activity

Repeated ECS significantly increased spontaneous locomotor activity measured 24 hr after the final shock. Fenfluramine, a 5-HT agonist, injected at the dose of 5 mg/kg did not affect spontaneous locomotor activity in control or ECS rats. Fenfluramine at the dose of 10 mg/kg and an other 5-HT agonist, m-CPP, at the dose of 5 mg/kg, reduced locomotor activity by about 70% in both groups. The serotonergic antagonists metergoline (1 mg/kg) and cyproheptadine (2 mg/kg) at these doses did not affect locomotor activity in control rats but significantly potentiated the ECS-induced increase. Metergoline 5 mg/kg, reduced locomotor activity in control rats but significantly enhanced the ECS-induced increase.

5-HT and 5-HIAA Concentrations

Brain concentrations of 5-HT were unchanged 0.5, 1 and

TABLE 2

EFFECT OF SEROTONINERGIC AGENTS ON THE INCREASED LOCOMOTOR ACTIVITY AFTER REPEATED ECS

Treatment	Dose mg/kg	Motility Counts (Mean ± SEM) Control	ECS
Vehicle	—	161 ± 18	248 ± 20
Fenfluramine	5	102 ± 16	228 ± 21
Fenfluramine	10	45 ± 9†	68 ± 10†
mCPP	5	33 ± 7†	57 ± 11†
Metergoline	1	115 ± 15	445 ± 28†
Metergoline	5	87 ± 9*	319 ± 29
Cyproheptadine	2	120 ± 10	309 ± 21*

All serotoninergic agents were administered IP 30 min before test. Locomotor activity was measured over 60 min (8–10 rats in each group). Significantly different from controls (vehicle).
*$p<0.05$.
†$p<0.001$.

TABLE 3

EFFECT OF SINGLE AND REPEATED ECS ON 5-HT AND 5-HIAA CONCENTRATIONS IN RAT BRAIN

Time After The Last ECS	5-HT		5-HIAA	
	Single ECS	Repeated ECS	Single ECS	Repeated ECS
Control	360 ± 11 (12)	345 ± 6 (12)	450 ± 22 (11)	432 ± 5 (12)
0.5 hr	339 ± 7 (7)	408 ± 14 (7)*	485 ± 13 (7)	502 ± 21 (7)†
1 hr	365 ± 7 (7)	370 ± 12 (7)	451 ± 7 (7)	473 ± 11 (7)†
24 hr	381 ± 13 (6)	328 ± 9 (6)	427 ± 11 (6)	499 ± 16 (6)†
5 days		350 ± 11 (6)		516 ± 24 (6)†
10 days		348 ± 6 (6)		524 ± 24 (6)†

Results are expressed in ng/g wet weight (mean ± SEM) with number of determinations in brackets. Significantly different from controls.
*$p<0.05$.
†$p<0.01$.

24 hr after a single ECS and 1 hr, 24 hr, 5 days and 10 days after repeated ECS. 0.5 hr after seven shocks 5-HT was elevated by 16%. 5-HIAA concentrations were not changed 0.5, 1 and 24 hr after a single ECS but they were about 12–19% elevated 0.5 hr, 1 hr, 24 hr, 5 and 10 days after repeated ECS.

DISCUSSION

Several studies have shown that serotonergic drugs produced head twitches in mice and rats probably by stimulation of central 5-HT receptors [4, 19, 22]. Recently it has been shown that selective inhibition of 5-HT_2 receptors with pirenperone inhibits 5-HT-mediated behavior, suggesting that this effect is predominantly mediated by 5-HT_2 receptors [11].

Our data indicate that single and repeated ECS have significant effects on functional activity of the serotonergic system. Single and repeated ECS depressed the number of head twitches induced by the presynaptically acting 5-HT agonist LiCl [31] given 1 hr after the last shock. This effect was not observed 24 hr after the last shock. Single and repeated ECS had no influence on head twitches induced by 5-HTP or 5-MT injected 1 or 24 hr after the last shock.

These data partially confirm the behavioral findings of Lebrecht and Nowak [17], that single and repeated ECS had no effect on head twitches induced by 5-HT or 5-HTP given 1 hr after the last shock but significantly increased the head twitches induced by these serotoninomimetics injected 24 hr after repeated ECS. In the present experiments repeated ECS had a potentiating effect on head twitches induced by 5-HTP, LiCl and 5-MT 5 and 10 days after cessation of a series of ECS. These differences may result from the different numbers of ECS used in our and the Lebrecht and Nowak [17] experiments (7 versus 10 shocks).

It has recently been reported that repeated ECS significantly enhanced spontaneous locomotor activity, for up to 5 days after the final shock [29]. This effect of ECS was potentiated by serotonergic blockers (metergoline, cyproheptadine) given 24 hr after the last shock and attenuated by 5-HT agonists. The enhanced locomotor activity induced by both serotonergic blockers could be explained initially by augmentation of serotonergic transmission. However, these results cannot be simply related to the 5-HT system, since

locomotor activity seems to be connected more to central dopaminergic than serotonergic mechanisms [16,18]. It has been reported that serotonergic agonists attenuated [5,28] or augmented dopamine-stimulated locomotor activity [20].

An inhibitory role of the serotonergic projection from the raphe for locomotor activity seems to be supported by the finding that lesions of the raphe resulted in hyperactivity [9] which was attenuated by serotonin administration [5]. The excitatory role of serotonergic neurons on locomotor activity and hyperactivity induced by d-amphetamine or ergometrine injected bilaterally into the nucleus accumbens has been observed after administration of a specific 5-HT_1 receptor agonist, Ru 24969 [13,20]. These results suggest that the serotonergic system, in different ways, modulates DA-stimulated locomotor activity. An increase in the locomotor activity produced by the 5-HT_1 agonist Ru 24969 in intact rats [20] and by 5-HT_2 antagonists (metergoline, cyproheptadine) in rats treated with repeated ECS (present results) suggests that ECS may enhance the serotonin input to dopamine neurons by 5HT_1 (activatory) and 5-HT_2 (inhibitory) receptors.

These results are in agreement with the effect of metergoline on tranylcypromine (monoamine oxidase inhibitor) and DOPA or tryptophan syndromes in which it converts the retropulsive "breaking" components (hind limb abduction; forepaw treading) into forward running behavior mediated by the dopaminergic system [7].

In binding experiments, Kellar *et al.* [15] and Vetulani *et al.* [25] reported that repeated ECS raised the density of 5-HT_2 but not 5-HT_1 receptors. Thus enhanced 5-HT-agonist induced head twitches and other 5-HT-mediated behavioral models [7] produced by repeated ECS appear to be mediated by 5-HT_2 receptors which seem to facilitate the response to serotonergic agonists and inhibit dopaminergic neurons.

Biochemical analysis showed that single ECS had no effect on 5-HT and 5-HIAA concentrations in the whole rat brain, 0.5, 1 and 24 hr after the single shock. On the other hand, repeated ECS consistently raised 5-HIAA concentration in the brain up to 10 days after the last shock and increased brain 5-HT concentration only 0.5 hr after the final shock. These lasting metabolic effects of repeated ECS seem to correlate with the behavioral changes detected 5 and 10 days after the cessation of ECS but not with early behavioral changes. Therefore, one may postulate another mechanism of action of ECS in rats. One and 24 hr after repeated ECS the dopamine-mediated behavioral responses were very strong, but 5 or 10 days after the last shock they had almost completely disappeared [29]. The serotonin-mediated behavioral responses remained unchanged 24 hr after the last shock, but were significantly enhanced after 5 and 10 days. Thus, the disturbance in the balance between dopaminergic and serotonergic systems may tentatively explain the effects. In conclusion, we have shown that repeated ECS facilitates the response to serotonergic stimulation probably via activation of 5-HT_2 receptors which presumably inhibit dopaminergic neurons.

REFERENCES

1. Barton, J. L. ECT in depression. The evidence of controlled studies. *Biol Psychiatry* **12:** 687–695, 1977.
2. Bergstrom, D. A. and K. J. Kellar. Effect of electroconvulsive shock on monoaminergic receptor binding sites in rat brain. *Nature* **278:** 464–466, 1979.
3. Chiodo, L. A. and S. M. Antelman. Electroconvulsive shock: progressive dopamine autoreceptor subsensitivity independent of repeated treatment. *Science* **210:** 799–801, 1980.
4. Corne, S. J., R. W. Pickering and B. T. Warner. A method for assessing the effects of drugs on the central actions of 5-hydroxytryptamine. *Br J Pharmacol* **20:** 106–120, 1963.
5. Costall, B., R. J. Naylor, C. B. Marsden and C. J. Pycock. Serotoninergic modulation of dopamine response from the nucleus accumbens. *J Pharm Pharmacol* **28:** 523–526, 1976.
6. Curzon, C. and A. R. Green. Rapid method for determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of the brain. *Br J Pharmacol* **39:** 653–655, 1970.
7. Deakin, J. F. W. and A. R. Green. The effects of putative 5-hydroxytryptamine antagonists on the behaviour produced by administration of tranylcypromine and L-tryptophan or tranylcypromine and L-DDPA to rats. *Br J Pharmacol* **64:** 201–209, 1978.
8. Denber, H. C. B. Pharmacotherapy of depression. In: *Psychopharmacological Treatment—Theory and Practice,* edited by H. C. B. Denber. New York: Marcel Dekker, 1975, pp. 121–134.
9. Geyer, M. A., A. Puerto, D. B. Menkes, D. S. Segel and A. J. Mandell. Behavioral studies following lesions of mesostriatal serotonergic pathways. *Brain Res* **106:** 257–270, 1976.
10. Gillespie, D. D., D. H. Manier and F. Sulser. Electroconvulsive treatment. Rapid subsensitivity of the norepinephrine receptor coupled adenylate cyclase system in brain linked to down-regulation of β-adrenergic receptors. *Commun Psychopharmacol* **3:** 191–195, 1979.
11. Green, A. R., K. O'Shaughnessy, M. Hammond, M. Schächter and D. G. Grahame-Smith. Inhibition of 5-hydroxytryptamine mediated behaviour by the putative 5-HT_2 antagonist pirenperone. *Neuropharmacology* **22:** 573–578, 1983.
12. Heal, D. J., H. Akagi, J. M. Bowdler and A. R. Green. Repeated electroconvulsive shock attenuates clonidine-induced hypoactivity in rodents. *Eur J Pharmacol* **75:** 231–237, 1981.
13. Hunt, P. J. and L. Oberlander. The interaction of indole derivatives with the serotonin receptor and nondopominergic circling behaviour. In: *Serotonin-Current Aspects of Neurochemistry,* edited by B. Haber. New York: Plenum Press, 1981, pp. 547–562.
14. Kellar, K. J., C. S. Cascio, D. A. Bergstrom, J. A. Butler and P. Ladarola. Electroconvulsive shock and reserpine: effect on β-adrenergic receptors in rat brain. *J Neurochem* **37:** 830–836, 1981.
15. Kellar, K. J., C. S. Cascio, J. A. Butler and R. N. Kurtzke. Differential effects of electroconvulsive shock and antidepressant drugs on serotonin$_2$ receptors in brain. *Eur J Pharmacol* **69:** 515–518, 1981.
16. Kelly, P. H. and S. D. Iversen. Selective 6-OHOH induced destruction of mesolimbic dopamine neurons abolition psychostimulant induced locomotor activity in rats. *Eur J Pharmacol* **40:** 45–56, 1976.
17. Lebrecht, U. and J. Z. Nowak. Effect of single and repeated electroconvulsive shock on serotonergic system in rat brain—II behavioural studies. *Neuropharmacology* **19:** 1055–1061, 1980.
18. Nadaud, D., J. P. Herman, H. Simon and M. Le Moal. Functional recovery following transplantation of ventral mesencephalic cells in rat subjected to 6-OHDA lesions of mesolimbic dopaminergic neurons. *Brain Res* **304:** 137–141, 1984.
19. Nakamura, M. and H. Fukushima. Effect of 5,6-dihydroxytryptamine on head twitches induced by 5-HTP, 5-HT, mescaline and fludiazepam in mice. *J Pharm Pharmacol* **30:** 56–58, 1978.
20. Oberlander, L. Effects of a potent 5-HT agonist, Ru 24969, on mesocorticolimbic and nigrostrial dopamine systems. *Br J Pharmacol* **80:** 675 P, 1983.
21. Pilc, A. and J. Vetulani. Depression by chronic electroconvulsive treatment of clonidine hypothermia and (^{3}H)-clonidine binding to rat cortical membranes. *Eur J Pharmacol* **80:** 109–113, 1982.

22. Przegalinski, E., I. Żebrowska-Łupina, A. Wójcik and Z. Kleinrok. 5-Methoxytryptamine-induced head twitches in rats. *Pol J Pharmacol Pharm* **29**: 253–261, 1977.
23. Vetulani, J. Adaptive changes as the mode of action of antidepressant treatments. In: *Typical and Atypical Antidepressants Molecular Mechanisms*, edited by E. Costa and G. Racagni. New York: Raven Press, 1982, pp. 27–36.
24. Vetulani, J. Changes in responsiveness of central aminergic structures after chronic ECS. In: *ECT: Basic Mechanisms*, edited by B. Berer, R. D. Weiner and R. H. Belmaker. London: John Libbey et Co., 1983, pp. 33–45.
25. Vetulani, J., U. Lebrecht and A. Pilc. Enhancement of responsiveness of the central serotonergic system and serotonin-2 receptor density in rat frontal cortex by electroconvulsive treatment. *Eur J Pharmacol* **76**: 81–85, 1981.
26. Vetulani, J., A. Pilc, L. Antkiewicz-Michaluk, K. Golembiowska-Nikitin, U. Lebrecht and A. Rokosz. Adaptive changes of monoaminergic systems in rats treated chronically with imipramine, electroshock or serotoninomimetics. In: *Neuronal Plasticity and Memory Formation*, edited by C. Ajmone Marsan and H. Matties. New York: Raven Press, 1982, pp. 405–425.
27. Vetulani, J., R. J. Stawarz, J. V. Dingell and F. Sulser. A possible common mechanism of action of antidepressant treatments: Reduction in the sensitivity of the noradrenergic cyclic AMP generating system in the rat limbic forebrain. *Naunyn Schmiedebergs Arch Pharmacol* **293**: 109–114, 1976.
28. Warbritton, J. D., R. M. Stewart and R. J. Baldessarini. Decreased locomotor activity and attenuation of amphetamine hyperactivity with intraventricular infusion of serotonin rat. *Brain Res* **143**: 373–382, 1978.
29. Wielosz, M. Increased sensitivity to dopaminergic agonists after repeated electroconvulsive shock (ECS) in rats. *Neuropharmacology* **20**: 941–945, 1981.
30. Wielosz, M. Effects of electroconvulsive shock on monoaminergic systems in the rat brain. *Pol J Pharmacol Pharm* **35**: 127–130, 1983.
31. Wielosz, M. and Z. Kleinrok. Lithium-induced head twitches in rats. *J Pharm Pharmacol* **31**: 410–411, 1979.