Pharmacology Biochemistry & Behavior, Vol. 38, pp. 695–697. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

BRIEF COMMUNICATION

Bright Light Does Not Alter Muscarinic Receptor Binding Parameters[1,2]

MONIQUE L. GIROUX

Medical Scientist Training Program, The Ohio State University, College of Medicine

EWA MALATYNSKA

Department of Pharmacology, University of Arizona, Tucson, AZ

AND

STEVEN C. DILSAVER[3]

Department of Psychiatry and Behavioral Science
P.O. Box 20708, University of Texas, Houston, TX 77225

Received 18 June 1990

GIROUX, M. L., E. MALATYNSKA AND S. C. DILSAVER. *Bright light does not alter muscarinic receptor binding parameters.* PHARMACOL BIOCHEM BEHAV **38**(3) 695–697, 1991.—Seasonal Affective Disorders (SADs) are disorders of mood characterized by recurrent episodes of illness with a fixed relationship to season. Winter depression is characterized by recurrent onset of depression in the fall or winter followed by spontaneous recovery in the spring. This syndrome is responsive to treatment with bright light. The pathophysiology of depressive disorders may involve central muscarinic mechanisms. This possibility led to a series of physiological studies. The authors now report that contrary to expectation, treatment with bright light did not decrease the density of muscarinic receptors in either the hypothalamus or striatum.

Affective disorders Bright light Depression Muscarinic receptors

SEASONAL Affective Disorder (SAD) is characterized by a recurrent disturbance of mood at the same time annually (8). Winter depression is the most widely recognized form of SAD. Daily treatment with bright artificial light is the standard treatment for this condition. Factors mediating its efficacy are yet to be established.

Research suggests that inappropriate activation of muscarinic mechanisms partially mediates vulnerability to depressive illness (4,8). Recent studies indicate that bright light blunts the hypothermic response to the muscarinic agonist oxotremorine in both the Sprague-Dawley and Flinder's Sensitivity Line (FSL) (13). The FSL is endowed with a genetically transmitted supersensitivity to oxotremorine. The magnitude of the effect of treatment with bright light is greater in the FSL than the Flinder's Resistant Line (FRL). The latter is the FSL's control line. The FRL and Sprague-Dawley are difficult to distinguish.

The finding that bright light has a greater effect on the FSL than the FRL is consistent with our data base. The effect of bright light is maximized in the Sprague-Dawley by experimentally enhancing its responsiveness to oxotremorine as a consequence of chronic treatment with a muscarinic receptor antagonist or inescapable stress (5).

Bright light may affect decreased responsiveness to oxotremorine by altering the density of muscarinic receptors. Lomax and Jenden (10) demonstrated that the oxotremorine-induced hypothermia is due to its effect on a hypothalamic center. The retinohypothalamic pathway provides a link between retinal photoceptors and the hypothalamus (12). We now report the results of a study designed to measure the effects of chronic treatment with bright light during the regular photoperiod on the density and affinity of hypothalamic and striatal muscarinic receptors.

METHOD

Male Sprague-Dawley rats weighing 200–220 g at the beginning of the study were exposed to 7,400 lux full spectrum bright

[1]This work was conducted at The Ohio State University, in the Department of Psychiatry and Psychopharmacology Program.
[2]Supported by MH-005503-04 and the State of Ohio Neuroscience Program.
[3]Requests for reprints should be addressed to Steven C. Dilsaver.

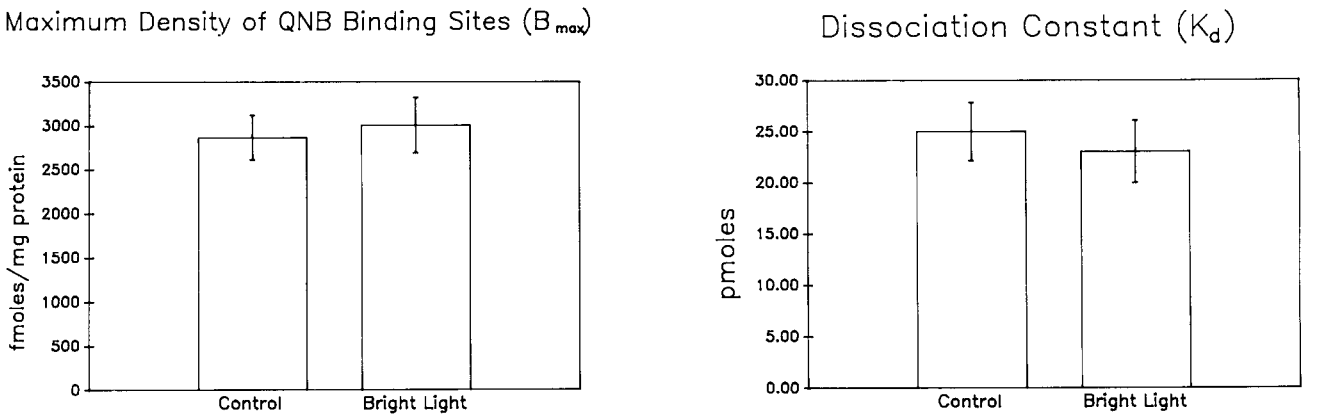

FIG. 1. This illustrates the lack of effect of bright light on muscarinic receptor density and affinity in the rat hypothalamus.

light from 6:00 a.m. to 6:00 p.m. for 7 consecutive days. Control animals were housed in the same room but were not exposed to bright light. The animals were sacrificed 12 hours after the last treatment. Their brains were removed and the striatum and hypothalamus were rapidly dissected on ice. Tissue samples were frozen at −70°C until binding was done.

Specific binding of [^{3}H]-quinuclidinylbenzilate (QNB) to muscarinic receptors was determined by the rapid filtration method of Watson et al. (16,17). Ten percent (10%) homogenates were prepared in 10 mM sodium-potassium-phosphate buffer (8.1 Mm Na_2HPO_4, 1.9 Mm KH_2PO_4; pH 7.4) with a Brinkman polytron (15 s, setting 5). This was followed by centrifugation for 10 min at 17,500 × g. The supernatant was discarded and the pellet rehomogenized in an equal volume of buffer, and recentrifuged. This was followed by a final homogenation. The pellet was diluted with buffer to yield a final homogenate of 1%. Aliquots (100 μl) of homogenate were added to yield concentrations of [^{3}H]-QNB of 0.10, 0.20, 0.40, 0.80, 1.20, 1.40, 2.0 and 4.0 nM in a total volume of 1.0 ml. These concentrations are sufficient to saturate all QNB binding sites. This mixture was incubated at 37°C for one hour. The assay was terminated by vacuum filtration over glass fiber filters (Whatman GF/B) using a Brandel cell harvester. Filters were washed three times with 3 ml of ice-cold 0.9% NaCl and counted in 10 ml of scintillation fluid. Filters were presoaked in 0.1% aqueous polyethyleneamine to minimize nonspecific binding to the filter. Specific binding was determined by the addition of 1 μM atropine sulfate and was less than 10%. All assays were done in triplicate.

Data Analysis

The dependent variable in this experiment is the difference in [^{3}H]-QNB binding to muscarinic receptors as a result of treatment with bright light. Receptor affinity (K_d) and maximum density of (B_{max}) were determined by a nonlinear curve fitting program, LIGAND (11). Data were analyzed using one- and two-site binding models according to the Law of Mass Action.

Data are expressed as sample means ± the standard error of the mean (SEM). The Shapiro-Wilk test was used to test for the normality of the distribution of dependent variables (K_d and B_{max}). The one-way analysis of variance (ANOVA) for independent data was used to assess significance of a difference between groups. A power analysis was used to determine the probability of a type II error (1).

RESULTS

Binding Data Using Hypothalamic Tissue

The Shapiro-Wilk's test indicated that the B_{max} and K_d were normally distributed. Total muscarinic receptor density (B_{max}) and receptor affinity (K_d) in the hypothalamus of the control group (N = 15) were 2,868 ± 256 fmol/mg protein and 24.98 ± 2.85 pmol, respectively. B_{max} and K_d values in the rats treated with bright light (N = 15) were 3,011 ± 317 fmol/mg protein and 23.05 ± 3.03 pmol, respectively. The means of B_{max}, $F(28) = 0.12$, n.s. and K_d, $F(28) = 1.99$, n.s., did not differ between the control and experimental groups. Correlation coefficients for the probability plot were .884 and .89 for B_{max} and .928 and .958 for K_d in control and bright light groups, respectively. The power of the one-way ANOVA used to determine whether B_{max} and K_d differed between groups were 96 and 95%, respectively. Figure 1 pictorially presents the negligible difference in binding parameters in hypothalamic tissue between the control and experiment groups.

Binding Data Using Striatal Tissue

The Shapiro-Wilks test indicated that B_{max} and K_d for the binding of QNB were normally distributed (14). B_{max} and K_d were 8076 ± 1478 fmol/mg protein (N = 6) and 35.52 ± 7.83 pmol, respectively in striatal tissue of the animals subjected to control conditions. The corresponding values for B_{max} and K_d in striatal tissue from the light-treated group were 7976 ± 985 fmol/mg protein and 37.66 ± 8.68 pmol, respectively. The differences in B_{max}, $F(10) = 0$ and K_d, $F(10) = 0.67$, did not differ between groups. Correlation coefficients for striatal tissue obtained from the control and bright light-treated groups were .951 and .927 for B_{max} and .837 and .851 K_d, respectively. The power of the parametric test used to determine whether B_{max} and K_d differed were 96 and 95%, respectively.

Figure 2 illustrates that B_{max} and K_d are essentially the same in striatal tissue obtained from the control and bright light-treated groups.

DISCUSSION

Bright light is the only somatic treatment for depression known to produce subsensitivity to muscarinic agonists (5). We chose to measure binding parameters in the hypothalamus because 1) oxotremorine-induced hypothermia is mediated via an effect on the hypothalamus, 2) treatment with bright light attenuates oxotremorine-induced hypothermia or blocks induction of supersensitivity to the thermic effects of this agonist (5) and 3) there is an anatomic link between retinal photoceptors and the hypothalamus (12). However, in this particular study, treatment with bright light did not alter the density of muscarinic receptors or their affinity for QNB in either the hypothalamus or striatum. These findings are particularly noteworthy.

The capacity of bright light to alter binding parameters cannot

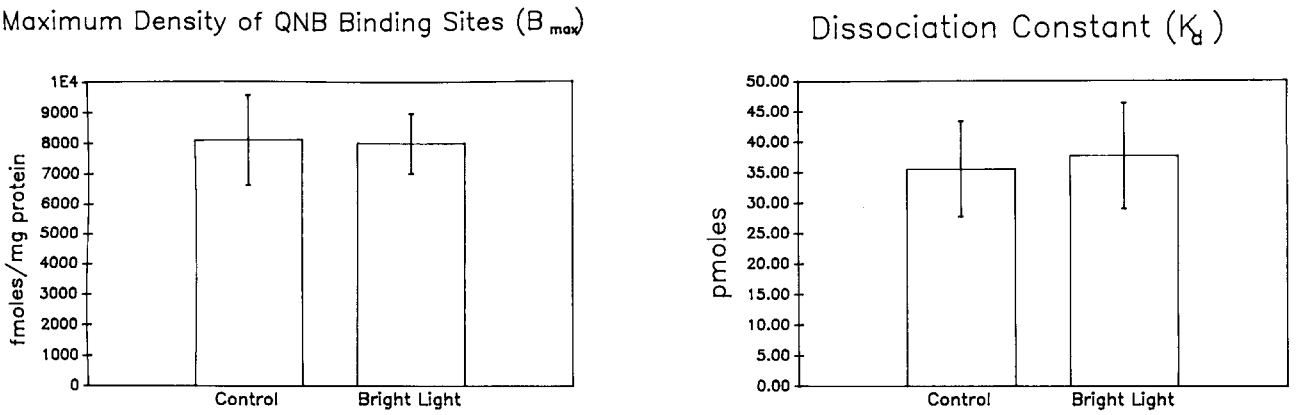

FIG. 2. This pictorially illustrates the lack of effect of treatment with bright light on muscarinic receptor density and affinity in rat striatum.

be categorically ruled out by our data. Some effects of bright light in the rat are contingent on the producing phase shifts (5). Treatment with bright light during the regular photoperiod does not produce such a shift in circadian phase. Receptor alterations cannot be ruled out at this time.

Methods of measuring binding parameters may also be critical. The homogenate binding technique used in this study detects an overall difference between regions tested or treatment conditions. The hypothalamus and the striatum are not homogeneous entities. Differences within specific foci in these structures may be masked by this technique. Receptor autoradiography may prove to be more sensitive in detecting regional differences in receptor level or affinity within a structure (9). Autoradiography also presents the advantage of simultaneously measuring differences in binding parameters in many brain areas. Muscarinic receptors are also heterogeneous and are divided into subtypes (15–18). Utilizing a muscarinic receptor antagonist, [^{3}H]-pirenzepine (PZ), autoradiography and homogenate binding studies (15,16) identified a distribution of binding sites representing a subpopulation of [^{3}H]-QNB binding sites. This subpopulation corresponds to the M1 receptor subtype. It is possible that more selective ligands, such as PZ, may yield differences not found with [^{3}H]-QNB.

The negative finding reported here may be more interesting than the positive result we anticipated. Changes in physiological measures may be mediated without changes in receptor binding parameters. Presynaptic events (such as change in the sensitivity of autoreceptors) (4), mechanisms coupling receptor and second messenger or alteration in the generation or efficacy of second messenger (3, 4, 6, 7) changes in the physico-chemical properties of neuronal membranes (2) and alterations in the functional properties of ion channels are possible means through which bright light may act upon muscarinic cholinergic systems.

REFERENCES

1. Armitage, P.; Berry, G. Statistical methods in medical resarch. Oxford: Blackwell Scientific Publications; 1987:181.
2. Baron, B.; Kloog, Y.; Wise, G. S. Fatty acid incorporation increases the affinity of muscarinic cholinergic receptors for agonists. Biochim. Biophys. Acta 801:342; 1984.
3. Berridge, M. J. Inositol triphosphate and diacylglycerol as second messengers. Biochem. J. 220:345–360; 1984.
4. Dilsaver, S. C. Pathophysiology of "cholinoceptive supersensitivity" in affective disorders. Biol. Psychiatry 21:813–829; 1986.
5. Dilsaver, S. C. Neurobiological effects of bright artificial light. Brain Res. Rev. 14:311–333; 1989.
6. Downes, P. C. Inositol phospholipids and neurotransmitter-receptor signalling mechanisms. Trends Neurosci. 6:313–316; 1983.
7. Fisher, S. K. Inositol lipids and signal transduction at CNS muscarinic receptors. Trends Pharmacol. Sci. Suppl. 7:61–65; 1986.
8. Janowsky, D. S.; El-Yousef, M. K.; Davis, J. M.; Sekerke, H. J. A cholinergic-adrenergic hypothesis of mania and depression. Lancet 2:632–635; 1972.
9. Kuhar, M. J.; Yamamura, H. I. Localization of cholinergic muscarinic receptors in rat brain by light microscopic autoradiography. Brain Res. 110:229–243; 1976.
10. Lomax, P.; Jenden, D. J. Hypothermia following systematic and intracerebral injection of oxotremorine in the rat. Neuropharmacology 5:353–359; 1966.
11. McPherson, G. A. A practical computer based approach to the analysis of radioligand binding experiments. Comput. Prog. Biomed. 12: 107–114; 1983.
12. Moore, R. Y.; Lenn, N. J. A retinohypothalamic projection in the rat. J. Comp. Neurol. 146:1–14; 1972.
13. Overstreet, D. H.; Dilsaver, S. C.; Janowsky, D. S.; Rezvani, A. H. Effects of bright light on responsiveness to a muscarinic agonist in rats selectively bred for endogenously increased cholinergic function. Psychiatry Res. 33:139–150; 1990.
14. Schlotzhauer, S. D.; Littell, R. C. Testing for normality. In: SAS system for elementary statistical analysis. Cary, NC: SAS Institute Inc.; 1987:117–125.
15. Spencer, D. G.; Harvath, E.; Traber, J. Direct autoradiographic determination of m_1 and m_2 muscarinic acetylcholine receptor distribution in the rat brain: Relation to cholinergic nuclei and projections. Brain Res. 380:59–68; 1986.
16. Watson, M.; Yamamura, H. I.; Roeske, W. A unique regulatory profile and regional distribution of [^{3}H]-pirenzipine binding in the rat provide evidence for distinct M1 and M2 muscarinic receptor subtypes. Life Sci. 32:3001–3011; 1983.
17. Watson, M.; Yamamura, H.; Roeske, W. [^{3}H]Pirenzipine and [^{3}H]quinuclidinyl benzilate binding to rat cerebral cortical and cardiac muscarinic binding to putative muscarinic subtypes. J. Pharmacol. Exp. Ther. 237:411–418; 1986.
18. Watson, M.; Roeske, T. W.; Vickroy, T. W.; Smith, T. L.; Akiyama, K.; Gulya, K.; Duckles, S. P.; Serra, M.; Adem, A.; Nordberg, A.; Gehlert, D. R.; Wamsley, J. K.; Yamamura, H. I. Biochemical and functional basis of putative muscarinic receptor subtypes and its implication. Trends Pharmacol. Sci. (Suppl.) 7:46–55; 1986.