Biochemical Pharmacology, Vol. 31, No. 19, pp. 3155-3157, 1982.
Printed in Great Britain.

0006-2952/82/193155-03$03.00/0

ALPRAZOLAM IN A BIOCHEMICAL MODEL OF DEPRESSION

Vimala H. Sethy and Donald H. Hodges, Jr.

CNS Research, The Upjohn Company, Kalamazoo, Michigan 49001 U.S.A.

(Received 27 April 1982; accepted 20 July 1982)

Reserpine produced depression in humans when it was used chronically for the treatment of hypertension [1]. Even today, reserpine-induced depression in animals is used as a model for screening antidepressant drugs [2]. Chronic treatment with reserpine increased the density of β-adrenergic receptors (β-AR) [3] and enhanced the ability of norepinephrine (NE) to stimulate the synthesis of cyclic adenosine-3'5' monophosphate [4]. On the other hand, chronic treatment with tricyclic antidepressants decreased the density of β-AR [5,6] and reduced the ability of NE to stimulate the net synthesis of cyclic adenosine-3'5' monophosphate [7]. Furthermore, it has been demonstrated that the reserpine-induced increase in β-AR is antagonized by repeated exposure of animals to electroconvulsive shock [3], a procedure utilized in the treatment of depression.

Alprazolam, a triazolobenzodiazepine, has been found to be an antidepressant in a double-blind placebo-controlled clinical study. In the same clinical investigation alprazolam was reported to be as potent as imipramine [8]. It is not known whether the antidepressant activity of alprazolam is mediated by β-AR or by some other mechanisms. This study describes the effects of chronic administration of alprazolam on reserpine-induced increases in β-AR in the cerebral cortex of rats. These results are compared with those obtained following administration of imipramine and diazepam under similar conditions.

Methods: Female Sprague-Dawley rats weighing 250-300 g were implanted with polyethylene cannulae in the jugular vein according to the method of Weeks [9]. Imipramine, alprazolam, diazepam (each 10 mg/kg/day) and reserpine (0.1 mg/kg/day) were infused chronically through the venous cannula for 15 minutes every hour on the hour for 2 or 3 weeks in a volume of 1.12 ml/day. Control rats received an equal volume of the vehicle (95% ethyl alcohol).

Administration of each drug was stopped immediately prior to sacrificing the animals. After decapitation, the brain was quickly removed from the skull and the cerebral cortex was dissected out. The tissue was frozen at -70° until assayed.

β-adrenergic receptor binding assays were carried out by the method of Bylund and Snyder [10] with slight modifications, as described previously [5], using [^{3}H]-dihydroalprenolol ([^{3}H]-DHA, sp. ac. 40.6 Ci/mmol) as a ligand in the range of 0.15 to 1.5 nM. Data were subjected to Scatchard analysis for the determination of dissociation constant (K_d) and maximum number of binding sites (B_{max}). Statistical analysis was done using the paired t-test.

Results and Discussion: Chronic treatment (2-3 weeks) with reserpine significantly ($p < 0.001$) increased the density of β-AR in the cerebral cortex (Tables 1 and 2) Imipramine

(2 weeks) significantly decreased the density of β-AR, as reported previously [11,12], and also reduced the reserpine-induced increase in β-AR receptors (Table 1).

Table 1. Effect of chronic (2 weeks) intravenous infusion of reserpine and imipramine on specific binding of [^{3}H]-DHA in the cerebral cortex of the rat

Treatment	Dose (mg/kg/day)	B_{max}* (fmol/mg protein)	K_d*(nM)
Vehicle	--	151 ± 4	0.73 ± 0.02
Imipramine	10	121 ± 7†	0.71 ± 0.04
Reserpine	0.1	193 ± 5‡	0.66 ± 0.04
Imipramine + Reserpine	10, 0.1	167 ± 5§	0.76 ± 0.05

*Mean± S.E. of four observations.
†p<0.003 compared to control.
‡p<0.001 compared to control.
§p<0.007 compared to reserpine.

Chronic treatment with alprazolam (2 and 3 weeks) and diazepam (3 weeks) had no significant effect on B_{max}. However alprazolam, when administered for 2 or 3 weeks, significantly ($p<0.001$ and 0.015, respectively) reduced the reserpine-induced increase in β-AR. Under similar conditions, diazepam had no significant effect on the reserpine-induced increase in the density of β-AR (Table 2). Chronic treatment with reserpine, imipramine, alprazolam and diazepam had no significant effect on the K_d of [^{3}H]-DHA binding to cortical tissue. At two weeks, alprazolam in combination with reserpine significantly ($p<0.007$) reduced the K_d of [^{3}H]-DHA binding to the cortex. However similar effects were not observed after 3 weeks of treatment with alprazolam plus reserpine. Therefore we have no explanation for the 2 week reduction in K_d at the present time.

Table 2. Effect of chronic intravenous infusion of reserpine, alprazolam and diazepam on specific binding of [^{3}H]-DHA in the cerebral cortex of the rat

		2 Weeks		3 Weeks	
Treatment	Dose (mg/kg/day)	B_{max}*(fmol/mg protein)	K_d*(nM)	B_{max}*(fmol/mg protein)	K_d*(nM)
Vehicle	--	152 ± 3	0.71 ± 0.02	145 ± 5	0.77 ± 0.05
Alprazolam	10	143 ± 4	0.66 ± 0.01	151 ± 9	0.91 ± 0.08
Diazepam	10	--	--	147 ± 11	0.78 ± 0.07
Reserpine	0.1	208 ± 3†	0.68 ± 0.02	224 ± 3†	0.82 ± 0.05
Alprazolam + Reserpine	10, 0.1	181 ± 5‡	0.62 ± 0.01	197 ± 5§	0.76 ± 0.04
Diazepam + Reserpine	10, 0.1	--	--	216 ± 3	0.75 ± 0.02

*Mean ± S.E. of 3-4 observations.
†p<0.001 compared to control.
‡p<0.001 compared to reserpine.
§p<0.015 compared to reserpine.

Unlike tricyclic antidepressants, alprazolam and bupropion do not alter the density of β-AR in normal rats [13]. However, in the reserpine-induced model of depression alprazolam, like imipramine, decreases to the same extent the drug-induced increase in the density of β-AR. These results indicate that β-AR may play an important role in antidepressant activity of alprazolam. Further work is in progress with atypical antidepressants like mianserin and bupropion using reserpine-induced increases in β-AR as a model of depression.

References

1. J. C. Muller, W. W. Pryor and J. E. Gibbons, *JAMA* 159, 836 (1955).
2. A. Barnett and R. I. Taber, in *Screening Methods in Pharmacology* (Eds. R. A. Turner and P. Hebborn), p. 209. Academic Press, New York (1971).
3. K. J. Keller, C. S. Cascio, D. A. Bergstrom, J. A. Butler and P. Iadarola, *J. Neurochem.* 37, 830 (1981).
4. J. B. Blumber, J. Vetulani, R. J. Stawarz and F. Sulser, *Eur. J. Pharmacol.* 37, 357 (1976).
5. V. H. Sethy and D. W. Harris, *Eur. J. Pharmacol.* 75, 53 (1981).
6. S. P. Banerjee, L. S. Kung, S. J. Riggi and S. K. Chanda, *Nature* 268, 455 (1977).
7. F. Sulser, J. Vetulani and P. L. Mobley, *Biochem. Pharmacol.* 27, 257 (1978).
8. L. F. Fabre and J. McLendon, *Curr. Ther. Res.* 27, 474 (1980).
9. J. R. Weeks, in *Methods in Psychobiology*, Vol. 2 (Ed. R. E. Myers), p. 155. Academic Press, New York (1972).
10. D. B. Bylund and S. H. Snyder, *Mol. Pharmacol.* 12, 568 (1976).
11. M. Asakura, T. Tsukamoto and K. Hasagawa, *Brain Res.* 235, 192 (1982).
12. D. A. Kendall, G. M. Stancel and S. J. Enna, *J. Neurosci.* 2, 354 (1982).
13. V. H. Sethy and D. W. Harris, *Drug Devel. Res.*, in press (1982).