Br. J. Pharmac. (1980), **71**, 359–361

THE INTERACTION OF TRAZODONE WITH RAT BRAIN MUSCARINIC CHOLINOCEPTORS

DEBORAH K. HYSLOP & DUNCAN P. TAYLOR

Biologic Research, Mead Johnson Pharmaceutical Division, Evansville, IN 47721, U.S.A.

The muscarinic receptor binding of trazodone, a new nontricyclic antidepressant, was compared with established tricyclic antidepressants. The ability to inhibit the binding of [^{3}H]-quinuclidinyl benzilate *in vitro* was used for comparing atropine-like effects. Trazodone was found to have essentially no activity at the muscarinic acetylcholine binding site in comparison to the tricyclic antidepressants.

Introduction Trazodone (Desyrel) is a new antidepressant agent which is therapeutically equivalent to the conventional tricyclic antidepressants (Kellams, Klapper & Small, 1979) but which significantly lacks the anticholinoceptor side effects of the tricyclic compounds (Gershon & Newton, 1980). It has been suggested that the inhibition of binding of tritiated muscarinic antagonists *in vitro* may be useful in estimating the potential for atropine-like effects *in vivo* (Snyder & Yamamura, 1977). Golds, Przyslo & Strange (1980) have described the interaction of some of the newer, 'atypical' antidepressants with muscarinic acetylcholine receptors using the displacement of [^{3}H]-atropine. We have used the inhibition of [^{3}H]-3-quinuclidinyl benzilate ([^{3}H]-QNB) binding *in vitro* to estimate the anticholinoceptor activity of trazodone and to confirm the findings of Golds *et al.* (1980) in our system.

Method The relative affinities of drugs for muscarinic cholinoceptor binding sites were evaluated on the basis of the ability to displace [^{3}H]-QNB from washed membranes obtained from rat hippocampi (Snyder & Yamamura, 1977). Male Sprague-Dawley rats were decapitated, the brains removed, and the hippocampi dissected and stored at −80°C until required. Pooled hippocampi were homogenized with a polytron, and membranes were recovered and washed once by centrifugation at 39,000 *g* for 10 min in 200 volumes of 50 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid-KOH, pH 7.4 (20°C). The washed membranes were resuspended in 20 volumes of ice-cold buffer. Binding was measured following incubation of 75 to 100 μg membrane protein in the presence of [^{3}H]-QNB (New England Nuclear, sp. act. = 29.4 Ci/mmol) and drug in duplicate for 90 min at 25°C. Specific binding amounted to 87% of total binding and was defined by the displacement of radioactivity in the presence of 10 μM atropine. Filtration and counting procedures have been described (Snyder & Yamamura, 1977). The concentration of drug which inhibited specific binding by 50% (the IC_{50}) and Hill coefficients were obtained from linear regression analysis of log-logit plots at five concentrations of drug each.

Results Saturation binding experiments with the ligand concentration ranging from 5.5×10^{-11} M to 4.5×10^{-9} M revealed a dissociation constant of $7.8 \times 10^{-11} \pm 0.5 \times 10^{-11}$ M (s.e. mean) or 7.2×10^{-11} M $\pm 0.7 \times 10^{-11}$ M (s.e. mean) for six individual preparations from the brains of female or male rats, respectively. There was no statistically significant difference between these results.

Various drugs were assayed for their ability to inhibit [^{3}H]-QNB binding, and the data obtained are given in Table 1. The tricyclic antidepressants possess from 0.001 to 0.005 the potency of atropine in this assay, while trazodone is at least two orders of magnitude weaker than the tricyclics (0.000007 the potency of atropine). The Hill coefficient for the interaction of trazodone with the [^{3}H]-QNB binding site was significantly greater than the value of unity obtained for atropine.

The greater-than-unity value for the Hill coefficient as well as the very large K_I suggested that trazodone might inhibit [^{3}H]-QNB binding via a nonspecific drug-membrane interaction. We conducted saturation binding experiments in the presence of various concentrations of trazodone. The results revealed a decrease in the maximum number of [^{3}H]-QNB binding sites resulting from increasing concentrations of trazodone. A purely competitive interaction at this binding site is thus ruled out.

Discussion The ability of tricyclic antidepressants to inhibit binding at muscarinic cholinoceptor sites as determined in this study is in agreement with earlier results from other laboratories (Snyder & Yamamura, 1977; Golds *et al.*, 1980). The values for the Hill coefficients of interaction for amitriptyline, desipramine,

0007-1188/80/140359-03 $01.00

Table 1 *In vitro* estimate of the anticholinergic activity of antidepressants

Drug	*Inhibition of [^{3}H]-QNB binding* K_I (nM)*	*Hill coefficient**	*Range of human plasma concentrations after chronic treatment* (nM)†
Amitriptyline	151 ± 83	0.84 ± 0.38	340–900
Clomipramine	146 ± 21‡	1.65 ± 0.23	—
Desipramine	456 ± 166	0.63 ± 0.06	140–610
Imipramine	94.1 ± 37.7	1.05 ± 0.19	470–790
Trazodone	75,200 ± 600‡	2.03 ± 0.28‡	1000–3100

* Values are $\bar{x}$ ± s.e. mean. For atropine, K_I = 0.54 ± 0.40 nM, Hill coefficient = 1.00 ± 0.13. Inhibition constants were obtained from the relationship $K_I = IC_{50}/1 + [L]/K_D$, where [L] = concentration of [^{3}H]-QNB (33 pm in these experiments) and K_D = dissociation constant for QNB.

† References to plasma concentrations of antidepressants: amitryptyline, Ziegler, Co, Taylor, Clayton & Biggs (1976); desipramine, Friedel, Veith, Bloom & Bielski (1979); imipramine, Glassman & Perel (1978); trazodone, Putzolu, Pecknold & Baiocchi (1976).

‡ $P < 0.05$ vs atropine (Student's t test).

and imipramine obtained by Golds *et al.*, (1980) were also supported.

The right-hand column of Table 1 reveals the range of equilibrium plasma concentrations of various antidepressants which give beneficial results when administered in a therapeutic regimen. All of the tricyclic antidepressants are beneficial over roughly the same concentration range, while the *in vivo* plasma concentration of trazodone which is beneficial is two to five times greater. Notably, the plasma concentrations of the tricyclic antidepressants are of the same order as, or even greater than the concentration found to inhibit cholinoceptor binding by 50% *in vitro*. In contrast, the plasma concentration for trazodone is at least an order of magnitude less than the IC_{50} *in vitro*. On the basis of these *in vitro* results it may be predicted that treatment with trazodone at levels providing beneficial effects would lack the anticholinergic side effects accociated with tricyclic antidepressant therapy. Double-blind clinical trials have revealed a significantly higher incidence of anticholinergic side effects for imipramine versus trazodone or placebo, while the side effects noted with trazodone were no more numerous or severe than those noted with the placebo (Gershon & Newton, 1980).

It has been suggested that the clinical efficacy of antidepressants may be attributed to their interaction with the muscarinic cholinoceptor system (Snyder & Yamamura, 1977). Trazodone does not inhibit muscarinic receptor ligand binding in a competitive manner, whereas the tricyclic antidepressants do (Golds *et al.*, 1980). The high concentration of trazodone required to inhibit [^{3}H]-QNB binding and the nature of the interaction at the binding site suggest that a nonspecific drug-membrane interaction similar to that observed with local anaesthetics occurred (Burgermeister, Klein, Nirenberg & Witkop, 1978). The nonspecific nature of the interaction of trazodone at the muscarinic acetylcholine binding site obtained *in vitro* coupled with trazodone's inability to prevent physostigmine-induced lethality *in vivo* (Taylor, Hyslop & Riblet, 1980) leads to the conclusion that trazodone achieves its antidepressant action by activity at biochemical and physical sites distinct from the cholinergic system. Hence, cholinergic mechanisms may have little influence on the pathophysiology and pharmacotherapy of depression.

References

BURGERMEISTER, W., KLEIN, W.L., NIRENBERG, M. & WITKOP, B. (1978). Comparative binding studies with cholinergic ligands and histrionicotoxin at muscarinic receptors of several cell lines. *Mol. Pharmac.*, **14**, 751–767.

FRIEDEL, R.O., VEITH, R.C., BLOOM, V. & BIELSKI, R.J. (1979). Desipramine plasma levels and clinical response in depressed outpatients. *Commun. Psychopharmac.*, **3**, 81–87.

GERSHON, S. & NEWTON, R. (1980). Lack of anticholinergic side effects with a new antidepressant—trazodone. *J. clin. Psychiat.*, **41**, 100–104.

GLASSMAN, A.H. & PEREL, J.M. (1978). Tricyclic blood levels and clinical outcome: A review of the art. In

Psychopharmacology: A Generation of Progress, ed. Lipton, M.A., DiMascio, A. & Killam, K.F. pp. 917–922. New York: Raven Press.

GOLDS, P.R., PRZYSLO, F.R. & STRANGE, P.G. (1980). The binding of some antidepressant drugs to brain muscarinic acetylcholine receptors. *Br. J. Pharmac.*, **68**, 541–549.

KELLAMS, J.J., KLAPPER, M.H. & SMALL, J.G. (1979). Trazodone, a new antidepressant: Efficacy and safety in endogenous depression. *J. clin. Psychiat.*, **40**, 390–395.

PUTZOLU, S., PECKNOLD, J.C. & BAIOCCHI, L. (1976). Trazodone: Clinical and biochemical studies. II. Blood levels and therapeutic responsiveness. *Psychopharmac. Bull.*, **12**, 40–41.

SNYDER, S.H. & YAMAMURA, H.I. (1977). Antidepressants and the muscarinic acetylcholine receptor. *Arch. gen. Psychiat.*, **34**, 236–239.

TAYLOR, D.P., HYSLOP, D.K. & RIBLET, L.A. (1980). Trazodone, a new non-tricyclic antidepressant without anticholinergic activity. *Biochem. Pharmac.*, **29**, 2149–2150.

ZIEGLER, U.E., CO, B.T., TAYLOR, J.R., CLAYTON, P.J. & BIGGS, J.T. (1976). Amitriptyline plasma levels and therapeutic response. *Clin. Pharmac. Ther.*, **19**, 795–801.

(Received August 18, 1980.)