Comparisons of the actions of high and low doses of the MAO inhibitor tranylcypromine on 5-HT\(_2\) binding sites in rat cortex

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Summary. Tranylcypromine (TCP) is a commercially available antidepressant drug, and recent literature reports suggest that high doses of this drug may be particularly effective in treating refractory depression. Down-regulation of 5-HT\(_2\) receptors in rat cortex is an effect produced after chronic administration of several antidepressants, and we have conducted a chronic study comparing low- and high-dose TCP in this regard. Male Sprague-Dawley rats were administered TCP (0.5 or 2.5 mg/kg/day) or vehicle (distilled water) via Alzet minipumps implanted subcutaneously in the dorsal thoracic area. Groups of rats were killed 4, 10 or 28 days after pump implantation and whole cortex was dissected out and utilized for preparation of a membrane fraction. Binding studies were performed with this fraction using \(^3\)H-ketanserin as the radioligand. Down-regulation (decrease in B\(_{\text{max}}\)) of the 5-HT\(_2\) binding site was observed in high-dose animals after 10 and 28 days but not after 4 days. Low-dose TCP had no effect on 5-HT\(_2\) densities at any time interval. The affinity of \(^3\)H-ketanserin for the 5-HT\(_2\) site was not affected by either dose at any time interval. These results suggest that down-regulation of the 5-HT\(_2\) site may contribute to the efficacy of high-dose TCP in the treatment of refractory depression.

Introduction

Tranylcypromine (TCP) is a nonselective monoamine oxidase (MAO) inhibitor that has been used for many years as an antidepressant. A dose of approximately 0.5 mg/kg/day of TCP is sufficient to produce a level of MAO inhibition normally required for antidepressant efficacy (Ferris et al., 1975; Robinson et al., 1978; Giller and Lieb, 1980; Giller et al., 1982). Amsterdam and Berwish (1989) reported that a much higher dose of TCP (90–170 mg, or approximately 1.3–2.4 mg/kg/day) was effective in treating refractory depressives. This treatment had been reported to be effective in earlier case reports (Robinson, 1983; Guze and Baxter, 1987; Pearlman, 1987).
5-HT$_2$ receptors have been implicated in the etiology of depression. They are down-regulated following chronic treatment with several antidepressants (Baker and Greenshaw, 1989; Eison et al., 1991; Lafaille et al., 1991) and they have been recently reported to be elevated in postmortem brain tissue from drug-free depressives (Yates et al., 1990). TCP, at doses much higher (on a mg/kg basis) than those used in the clinical setting, has been reported to cause 5-HT$_2$ receptor down-regulation (Kellar et al., 1981; Goodwin et al., 1984). The present study compares the effects of low (0.5 mg/kg/day)- and high (2.5 mg/kg/day)-dose TCP on 5-HT$_2$ receptor density and affinity in rat cortex.

**Methods**

**Animals**

Male Sprague-Dawley rats were implanted in the dorsal thoracic area with Alzet 2ML2 osmotic minipumps loaded to administer the following doses of drugs: TCP (0.5 mg/kg/day), TCP (2.5 mg/kg/day), or vehicle (distilled water). At time intervals of 4, 10, or 28 days groups of animals (8–10 per group) were sacrificed by decapitation and the brains removed. Whole cortex was dissected out and a membrane fraction prepared and employed for studying 5-HT$_2$ receptor number and affinity using $^3$H-ketanserin as the radioligand (Leysen et al., 1982). The rest of brain (whole brain minus cortex, hippocampus and striatum, which were removed for other neurochemical studies) was retained for analysis of brain amine concentrations by HPLC (Baker et al., 1987), monoamine oxidase (A and B) activity using a radiochemical procedure (Wurtman and Axelrod, 1963), and TCP levels by electron capture gas chromatography (Nazarali et al., 1987).

**Drugs**

TCP, mianserin, and $\beta$-phenethylamine (hydrochlorides) and 5-HT creatinine sulphate were obtained from Sigma Chemicals. $\beta$-[Ethyl-1-$^{14}$C]-phenethylamine hydrochloride (50.8 mCi/mmoll, 5-[2-$^{14}$C]-hydroxytryptamine binoxalate (54.7 mCi/mmol) and $^3$H-ketanserin hydrochloride (60.0 Ci/mmol) were purchased from NEN chemicals. Tris (hydroxymethyl)aminomethane and poly(ethylenimine) were obtained from Fischer Scientific and Aldrich Chemicals, respectively.

$^3$H-ketanserin binding

Eight point saturation curves were obtained using a modification of the method described by Eison et al. (1990). Displaceable $^3$H-ketanserin binding was determined from the difference between total binding and binding in the presence of 10 $\mu$M mianserin (Goodnough and Baker, 1993). Bmax and Kd values were determined from Scatchard analysis of the data. Protein content was determined by the method of Lowry et al. (1951).