FAILURE OF CIMETIDINE TO ANTAGONISE DOPAMINE-INDUCED SUPPRESSION OF PROLACTIN *IN VITRO*

It has been suggested that cimetidine, the histamine receptor (H_2) antagonist (Brimblecome, Duncan, Durant, Emmett, Ganellin & Parson, 1975), may cause hyperprolactinaemia and gynaecomastia during the long-term treatment of patients with peptic ulcers (Hall, 1976; Delle Fave, Tamburrano, De Magistris, Natoli, Santori, Carratu & Torsoli, 1977). Since significant gynaecomastia rarely accompanies hyperprolactinaemia (Thorner, Edwards, Hanker, Abraham & Besser, 1977) and oral administration of single therapeutic doses of cimetidine rarely increases prolactin levels in man (Majumdar, Thomson & Shaw, 1978; Burland, Gleadle, Lee & Rowley-Jones, 1978), the association of treatment with this drug and the reported complication seem tenous. It is not clear whether stress, such as that related to venepuncture, has been entirely excluded as a possible non-specific cause of the reported hyperprolactinaemia in the cimetidine treated patients.

However, parenteral administration of large doses of cimetidine, such as 400 mg given i.v. as a bolus injection, does increase serum prolactin (Carson & Ippoliti, 1977; Burland *et al.*, 1978), suggesting there may be a relationship between cimetidine blood levels, higher than normally achieved during oral therapy, and increased prolactin secretion. The reported ability of the dopamine agonist bromocriptine to block the elevation of prolactin has led to the suggestion that cimetidine may act as a dopamine antagonist blocking pituitary dopamine receptors, thus interfering with the action of dopamine as a prolactin inhibiting factor and producing hyperprolactinaemia (Burland *et al.*, 1978). In this case, cimetidine should release prolactin in a similar fashion to other known dopaminergic antagonists (MacLeod & Lehmeyer, 1974). However, since bromocriptine blocks prolactin secretion after all known stimuli, whether they act through dopamine receptors or not, inhibition of a prolactin secretogue effect by this agent cannot be taken by itself to indicate that the mechanisms involved in cimetidine stimulation of prolactin secretion necessarily involve pituitary dopamine receptors.

To test the hypothesis, we have studied *in vitro* the effects and interactions of metoclopramide, a specific dopamine antagonist (Dougan, Mearwick & Wade, 1974) and cimetidine, on the dopamine induced inhibition of prolactin secretion from perfused columns of isolated rat pituitary cells.

The pituitary cell columns were prepared by the method of Yeo, Thorner, Jones, Lowry & Bessir (1979). Anterior pituitary glands were obtained from five female Wistar rats weighing 210–230 g. Each anterior lobe was cut into six–eight pieces and placed in 10 ml Earle's balanced salt solution (EBSS) containing trypsin (0.25 g/100 ml) and dopamine (5×10^{-6} M). They were mechanically dispersed 4 times, for 20 min each time, at 37° in a 'Teflon' dispersal apparatus and were then centrifuged at 420 *g* for 40 min. After discarding the supernatant, the pituitary cells were resuspended and filtered through 100 μ nylon gauze and mixed with 0.5 gm Biogel P2 beads (200–400 mesh) which had been previously preswollen overnight in normal saline and then equilibrated with EBSS containing trypsin inhibitor (0.04 g/100 ml) and bovine serum albumin (BSA, 0.25 g/100 ml). The cells and Biogel were packed in a plastic column (0.9 × 1 cm) and perfused in a waterbath at 37°C at a flow rate of 0.4 ml/min with EBSS containing BSA (0.25 g/100 ml), penicillin (25 mU/ml) and streptomycin (25 μg/ml) and gassed with 95% O_2 and 5% CO_2. The test substances and saline alone were added from an automatic sampler and mixed with the perfusion medium in a ratio of 1:9. Fractions of 7.5 min were collected from the column eluate.

Metoclopramide (Beecham Research Laboratories), cimetidine (Smith, Kline & French Laboratories) and dopamine (5-hydroxytyramine, as the hydrochloride, Sigma) were dissolved in 0.9% saline containing 5 mg% ascorbic acid as anti-oxidant. Prolactin was measured in the cell column eluate using reagents provided by the National Institutes for Arthritis, Metabolism and Digestive Diseases—Rat Pituitary Hormone Program. The prolactin standard used was NIAMDD—Rat Prolactin-RP-1.

In Figure 1 the effects of metoclopramide and cimetidine on the inhibition of prolactin release induced by dopamine are compared. During perfusion with medium containing dopamine (5×10^{-6} M), prolactin secretion was rapidly suppressed and this continued as long as the dopamine was added to the medium. In the absence of dopamine, prolactin secretion was active. The addition of metoclopramide (1×10^{-8} M to 1×10^{-4} M) overcame the inhibitory effect of dopamine on prolactin secretion. However, prolactin was again suppressed when dopamine was reinfused alone. Cimetidine, however, added at the same concentrations as metoclopramide, failed to alter the inhibitory action of dopamine on prolactin secretion. During perfusion with dopamine plus cimetidine, prolactin remained suppressed and similar to when dopamine was infused alone. Thus unlike metoclopramide, cimetidine did not block the inhibitory action of dopamine on prolactin secretion.

The failure of cimetidine to block the dopamine inhibition of prolactin release in the isolated rat pituitary cell column indicates that cimetidine does not block pituitary dopamine receptors even at high con-

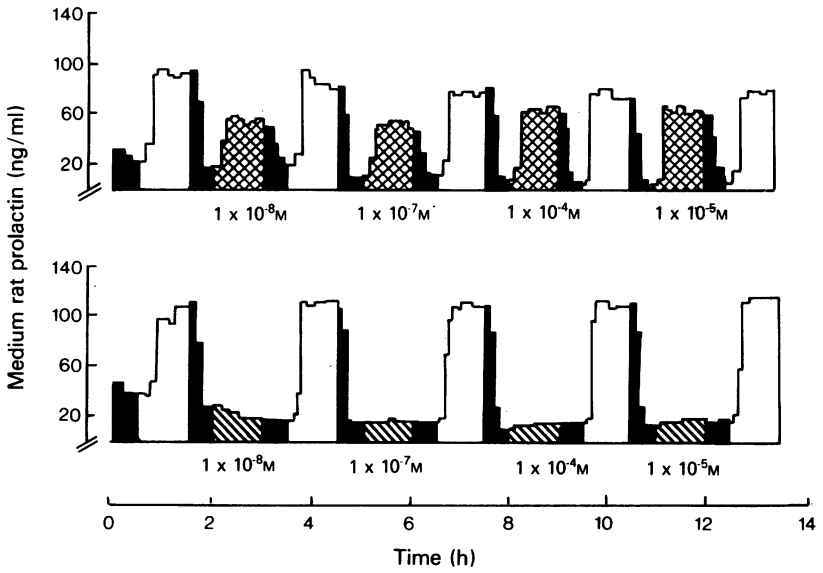

Figure 1 Rat prolactin concentrations in eluate from rat anterior pituitary cell columns exposed to pulses of medium containing dopamine (5×10^{-6} M) (■), dopamine (5×10^{-6} M) plus metoclopramide (1×10^{-8} M to 1×10^{-4} M) (▨), and dopamine (5×10^{-6} M) plus cimetidine (1×10^{-8} M to 1×10^{-4} M) (▧). The varying concentrations of metoclopramide and cimetidine are as shown. The blank bars represent prolactin secretion when saline alone was added to the perfusing medium.

centrations. In contrast, metoclopramide, a known dopamine receptor blocking agent and *in vivo* prolactin secretogue (McNeilly, Thorner, Volans & Besser, 1974; Delitala, Masala, Alagna & Devilla, 1976), blocked the dopamine induced inhibition of prolactin secretion by an action at the pituitary cell level. These observations suggest that the prolactin releasing activity of the H_2-receptor antagonist observed *in vivo* acts through mechanisms other than those involving pituitary dopamine receptors.

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ALTERED ELIMINATION OF DESMETHYLDIAZEPAM IN THE ELDERLY

Desmethyldiazepam (DD), the major metabolite of the widely used tranquilizer diazepam (D), is eliminated by hydroxylation to oxazepam (Schwartz, Koechlin, Postma, Palmer & Krol, 1965). After multiple dosing with D accumulation of DD occurs (Klotz, Antonin & Bieck, 1976). Since this metabolite possesses still considerable biological activity (Randall, Scheckel & Banzinger, 1965), DD will contribute to the clinical effects and untoward side effects of D.

Epidemiological data have indicated that central nervous side effects of D increased significantly with age (Boston Collaborative Surveillance Drug Program, 1973) and pharmacokinetic studies have demonstrated that the distribution of D was influenced by age (Klotz, Avant, Hoyumpa, Schenker & Wilkinson, 1975). In addition to these age-dependent alterations in the pharmacokinetics of D also age-dependent changes in the disposition of the biological active metabolite DD might contribute to the higher toxicity observed in older patients.

Therefore we compared in four young individuals (29–34 years) and in four elderly subjects (65–85 years) the disposition of DD following a single oral dose of 20 mg (Madar Notte, Ravizza, Milano). All subjects were male nonsmokers with normal liver function. Eight venous blood samples were drawn into heparinized tubes over 5 days. Concentrations of DD were assayed in the different plasma samples by a specific and sensitive gaschromatographic procedure (Klotz *et al.*, 1975). For comparison of the pharmacokinetic data the two-tailed Student's *t*-test was used with the minimal level of significance of $P < 0.05$.

In both groups maximal plasma concentrations between 150 and 250 ng/ml were achieved within 10 h following the single oral dose of 20 mg DD. Thereafter a monoexponential decline was observed (Figure 1). The slope of the regression line was much steeper in the younger individuals than in the older subjects. This resulted in a prolonged half-life ($T_{\frac{1}{2}}$) for the elderly. According to Westlake (1970) total plasma clearance (Cl) from the area under the curve and according to Gibaldi, Nagashima & Levy (1969) the apparent volume of distribution ($V_d\beta$) were calculated. All these parameters are compared in Table 1. The age-dependent prolongation in $T_{\frac{1}{2}}$ was due to a reduced Cl in the elderly, while plasma protein binding (97.6%) and $V_d\beta$ did not differ significantly.

Our results indicate that in man hepatic clearance of DD is influenced by age. Other benzodiazepines, like diazepam (eliminated primarily by demethylation; Klotz *et al.*, 1975), oxazepam (eliminated by glucuronidasation; Shull, Wilkinson, Johnson & Schenker, 1976) and lorazepam (eliminated also by glucuronidasation; Kraus, Desmond, Marshall, Johnson, Schenker & Wilkinson, 1978) are cleared independent of age. However, chlordiazepoxide which is in part also eliminated by hydroxylation (like DD)

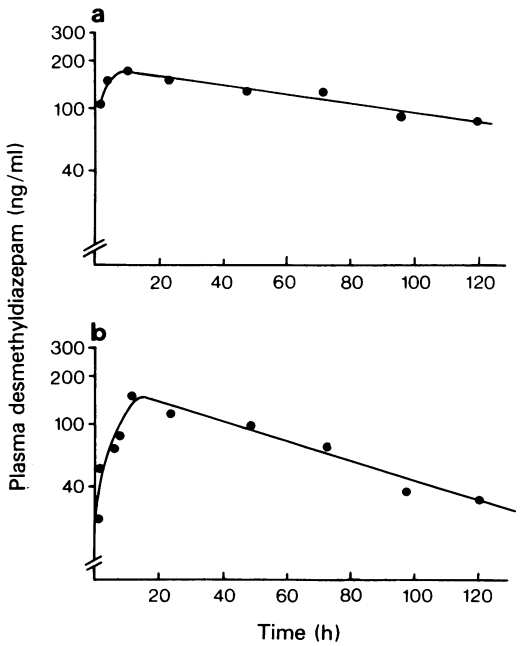

Figure 1 Plasma concentration-time profiles of desmethyldiazepam after a single oral dose of 20 mg in one elderly subject (a, 74 years, $T_{\frac{1}{2}}\beta = 102$ h, Cl = 6.1 ml/min), and one young individual (b, 31 years, $T_{\frac{1}{2}}\beta = 56.8$ h, Cl = 13.7 ml/min).