# Effects of desipramine and reserpine on 'free' and 'bound' acetylcholine in rat brain

The possible involvement of central cholinergic mechanisms in the mode of action of tricyclic thymoleptics has been proposed by Biel, Nuhfer & others (1962), Ho, Freeman & others (1966) and Benešová (1967). The imipramine-like drug desmethylimipramine (desipramine) is able to counteract both the sedative and the central acetylcholine-like effects of reserpine (Sulser, Bickel & Brodie, 1964). As reserpine alters the brain levels of acetylcholine (Giarman & Pepeu, 1962; Beani, Ledda & others, 1966; Malpica, Jurupe & Campos, 1970), tricyclic thymoleptics may antagonize the central acetylcholine-like effects of reserpine by influencing the levels of acetylcholine in brain tissue. We have examined the influence of desipramine on the levels of brain acetylcholine and determined whether this action could be related to the antagonism of the reserpine-induced central acetylcholine-like effects.

Male Sprague-Dawley rats, 180–220 g were used. Acetylcholine iodide, eserine sulphate and reserpine (3,4,5-trimethoxybenzoyl-reserpate) were supplied by Nutritional Biochemicals Corp. while desipramine HCl (Pertofrane) was kindly donated by Geigy (Canada) Ltd. All drugs and solvents (in controls) were injected intraperitoneally in a volume of 1 ml per 100 g. Desipramine was given in 0·9% NaCl. Reserpine, dissolved in $2 \times 10^{-2}$ M $H_3PO_3$ (0·1% w/v), was adjusted to pH 5 with N NaOH immediately before injection. Animals were killed by immersion in liquid nitrogen according to Takahashi & Aprison (1964) and, after decapitation, their brains (without olfactory bulbs, cerebellum and medulla) were rapidly excised, weighed and then homogenized in ice-cold saline containing eserine sulphate (15 $\mu$g ml$^{-1}$) and cupric chloride (17 $\mu$g ml$^{-1}$) to extract 'free' acetylcholine; the residue was extracted with acid-ethanol to obtain the 'bound' acetylcholine fraction (Crossland & Slater, 1968). The fractions of total brain acetylcholine so obtained are referred to as directly measured 'free' or 'bound' acetylcholine. In other experiments the 'total' and bound' acetylcholine respectively were obtained from pooled halves of brains of two animals. 'Total' acetylcholine was extracted according to Crossland (1961) and the 'bound' fraction was obtained as described above. In this case, the values for 'free' acetylcholine were calculated by subtracting the values of 'bound' fraction from those of 'total' acetylcholine.

The tissue extracts were bioassayed on the eserinized frog rectus (Hrdina & Maneckjee, 1971). The data were statistically analysed using Student's *t*-test.

1 h after administration of desipramine (10 mg kg$^{-1}$) there was significant decrease in the 'bound' acetylcholine fraction of the entire brain (Table 1) when compared with controls. The ratios 'free': 'bound' and 'free': 'total' acetylcholine were increased in desipramine-treated rats. After chronic administration of desipramine (10 mg kg$^{-1}$, twice a day for 7 days and animals being killed 18 h after the last dose) there was a significant increase in the calculated amount of 'free' acetylcholine only and consequently an increase in ratios 'free': 'bound' and 'free': 'total' acetylcholine (Table 1). During this experiment the animals were housed at 24° and were allowed free access to food and water. The weight of control rats increased by an average of 33·5 g (to 116 $\pm$ 3% of initial weight) while the weight of desipramine-treated rats decreased by an average of 6·2 g (to 93 $\pm$ 3% of initial weight).

Administration of reserpine (2·5 mg kg$^{-1}$) produced, after 4 h, a significant increase in the amount of 'bound' acetylcholine (Table 2). The ratios 'free': 'bound' and 'free': 'total' were decreased. At the time of death, the reserpine-treated animals displayed characteristic sedation, ptosis, miosis and diarrhoea.

No changes in 'free', 'bound' or 'total' brain acetylcholine were found at 4 h after the simultaneous administration of desipramine (10 mg kg$^{-1}$) and reserpine (2·5 mg

Table 1. *Effect of acute and chronic administration of desipramine on the concentration of 'free' and 'bound' acetylcholine (ACh) in rat brain.*

| Drug | 'Free' ACh | 'Bound' ACh nmol $g^{-1}$ ± s.e. | 'Total' ACh | Ratio (%) | | |
|---|---|---|---|---|---|---|
| | | | | F/T | F/B | B/T |
| None | 3·02 ± 0·48 (4) | 12·95 ± 1·01 (4) | 15 66 ± 1·13‡ (4) | 19·3 | 23·3 | 82·7 |
| Desipramine 10 mg $kg^{-1}$ 60 min | 3·39 ± 0·51 (6) | 10·94 ± 0·75* (6) | 14·32 ± 0·35‡ (6) | 23·7 | 31·0 | 76·4 |
| % change | + 12·3 | − 15·5 | −8·5 | +22·8 | +33 | −7·6 |
| None | 6·98 ± 0·45† (12) | 10·16 ± 0·48 (12) | 17·75 ± 0 48 (16) | 39·3 | 68·7 | 57·2 |
| Desipramine 10 mg 2 × day for 7 days | 8·86 ± 0·56*† (5) | 9·81 ± 0·57 (5) | 18·67 ± 0·67 (5) | 47·4 | 90·3 | 52·5 |
| % change | +26·9 | − 3·5 | + 5·2 | + 20·6 | + 31·4 | −8·2 |

* Significantly different compared with controls: $P < 0{\cdot}05$
F/T: 'free'/'total'; F/B: 'free'/'bound'; B/T: 'bound'/'total' acetylcholine.
† Calculated value obtained by subtracting the individual values for 'bound' ACh from the values of 'total' ACh.
‡ Calculated values obtained by adding the individual values for 'free' and 'bound' ACh.

$kg^{-1}$). The ratios also remained unaltered. In this circumstance, the ptosis, miosis and the muscular rigidity seen after reserpine alone were blocked, while the sedative effect of reserpine was only partially inhibited.

These results provide additional information about the possible mechanism of action of desipramine which we find is able to influence the amount and the ratio of 'free' and 'bound' acetylcholine fractions of rat brain. Acute administration of desipramine produced a modest, though significant decrease in the 'bound' acetylcholine fraction. As a result, both the ratio 'free':'bound' and 'free':'total' were increased. Chronic treatments with desipramine also resulted in an increase of ratios 'free':'bound' and 'free':'total' acetylcholine, although significant change was found only in the calculated amount of 'free' acetylcholine fraction. Reserpine significantly increased the amount of 'bound' acetylcholine in whole brain (by 19·8%) and both the ratio 'free':'bound' and 'free':'total' acetylcholine were decreased. These changes were contrary to those observed after acute administration of desi-

Table 2. *Effect of reserpine on the concentration of 'total and 'bound' acetylcholine in the whole brain of the rat.*

| Drug | 'Free' ACh† | 'Bound' ACh nmol $g^{-1}$ ± s.e. | 'Total' ACh | Ratio (%) | | |
|---|---|---|---|---|---|---|
| | | | | F/T | F/T | B/T |
| None | 6·98 ± 0·45 (12) | 10·16 ± 0·48 (12) | 17·75 ± 0·48 (16) | 39·3 | 68·7 | 57·2 |
| Reserpine 2·5 mg $kg^{-1}$ 4 h | 6·36 ± 0·95 (6) | 12·17 ± 0·58* (6) | 18·52 ± 0·70 (6) | 34·3 | 52·2 | 65·7 |
| % change | − 9 | + 19·8 | + 10·4 | − 12·7 | − 24·0 | + 14·9 |

* $P < 0{\cdot}025$.
† Calculated value obtained by subtracting the individual values for 'bound' ACh from the values of 'total' ACh.

pramine. When the two drugs were given concurrently, there was no change in the amount of 'bound' acetylcholine presumably because desipramine was able to counteract the reserpine-induced increase in this fraction. If the effects of reserpine and desipramine on brain acetylcholine levels reflect their interference with central cholinergic mechanisms, our observation may offer a possible explanation for the reported antagonism of desipramine to the central acetylcholine-like effects of reserpine (Sulser & others, 1964). Furthermore, our present findings of decreased 'bound' acetylcholine content after desipramine along with an earlier report on the lowering effect of a similar dose of desipramine on total acetylcholine in the striatum (Hrdina, Ling & Maneckjee, 1971) are compatible with the view (Cairncross, Gershon & Gust, 1962; Biel & others, 1962; Ho, & others, 1966; Benešová, 1967) that alteration of central cholinergic mechanisms may play a role in the mode of central action of imipramine-like drugs.

The identity and the physiological significance of 'free' and 'bound' brain acetylcholine fractions still remain unclear. According to Rogers & Slater (1971), the 'bound' acetylcholine might represent the stored neurohormone, while the 'free' fraction is probably a mixture of newly synthesized cytoplasmic acetylcholine, released neurohormone, and some 'bound' acetylcholine released during extraction. This could explain the variations in amounts of 'free' acetylcholine: Crossland & Slater (1968) 9·2–15%, Richter & Goldstein (1970) 25%, Milosevic (1970) 30%. Expressing the 'free' fraction as the difference between directly measured 'total' and 'bound' acetylcholine, we obtained 39·3%. This could be because of an inevitable loss, occurring during the preparation of the 'free' fraction, being added to the estimated value, whereas when 'free' acetylcholine is measured directly this loss is excluded.

Some centrally acting drugs can differentially influence the 'free' and 'bound' acetylcholine fractions in the brain (Crossland & Slater, 1968; Beani, Bianchi & others, 1969; Milosevic, 1970; Slater, 1971). Crossland & Slater (1968) suggested that drugs which differentially affect the 'free' and 'bound' fractions may so do by interfering with the synthesis, release and/or destruction of the putative neurotransmitter. Desipramine is known to interfere with the uptake (Glowinski & Axelrod, 1964; Fuxe & Ungerstedt, 1968), release (Brodie, Costa & others, 1968; Carlsson, Jonason & Lindquist, 1969) and turnover (Tarlov & Schildkraut, 1971) of noradrenaline and 5-HT in brain and peripheral nerve endings. It is therefore conceivable that desipramine may have produced the observed changes in brain acetylcholine fractions by (1) directly influencing the uptake of choline and storage of acetylcholine and/or (2) interfering with the synthesis and release of the putative neurotransmitter.

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# Antibacterial activity of phenylmercuric nitrate in zinc sulphate and adrenaline eye drops B.P.C. 1968

Recent determinations concerning the antibacterial activity of PMN and sodium metabisulphite mixtures in simple solution and in pharmaceutical preparations have been reported (Buckles, Brown & Porter, 1971; Richards & McBride, 1972; Richards & Reary, 1972; Richards, Fell & Butchart, 1972).

The antibacterial activity of PMN contained in zinc sulphate and adrenaline eye drops B.P.C. 1968 (19·8 ml) was examined after sterilization using autoclaving at 115–116° for 30 min; heating at 98–100° for 30 min; filtration using a Millipore membrane (size GSWP 02500, Millipore Limited, London). Killing times for the eye drops against *Pseudomonas aeruginosa* NCTC 7244 were determined at room temperature (20°) using a method similar to that of Richards & McBride (1971). After sterilization, 0·2 ml of an overnight culture containing approximately $5 \times 10^8$ bacteria $ml^{-1}$ was added to each bottle and 10, 20, 30, 45, 60, 90, 120, 150 and 180 min after, 1·0 ml samples were removed and added to 10 ml recovery medium consisting of thioglycollate (0·05% w/v) in nutrient broth (Oxoid No. 2). Incubation of the recovery medium was at 37° for 7 days. The concentration of PMN contained in the eye drops was determined before and after sterilization using the polarographic method of Porter (1968).

Killing times and polarographic determinations were also carried out for solutions of PMN (0·002% w/v) + sodium metabisulphite (0·1% w/v) and zinc sulphate and adrenaline eye drops similar to the B.P.C. 1968 formulation but not containing sodium metabisulphite.

Results (Table 1) show that the killing time for zinc sulphate and adrenaline eye drops B.P.C. 1968 varies depending on the sterilization method used. Filtration sterilized drops showed a more rapid killing effect than heat sterilized preparations. Although drops that had been heat sterilized, i.e. either by autoclaving 115–116° for 30 min or heating 98–100° for 30 min, showed a decrease in the concentration of PMN,