# Effect of chlorpromazine and some of its metabolites on the dopamine-sensitive adenylate cyclase of rat brain striatum

The neuroleptic drug chlorpromazine is subject to many metabolic modifications and several of the resulting metabolites have been detected in the urine and plasma of patients treated with the drug (Goldenberg & Fishman, 1972). The poor correlation found between plasma levels of chlorpromazine and its therapeutic effect in individual schizophrenic patients has led to the suggestion that some of its metabolites may be involved in its action (Curry, 1971; Curry, Lader & others, 1972).

It has been suggested that neuroleptic drugs such as chlorpromazine act by blocking central dopamine receptors (Matthysse, 1973). This results in a feedback-mediated increase in dopamine turnover and an accumulation of the dopamine metabolite homovanillic acid in those brain areas receiving a dopaminergic input (Andén, Roos & Werdinius, 1964). Dopamine receptor blockade also explains the ability of neuroleptics to antagonize amphetamine-induced stereotyped behaviour in rats, which probably results from stimulation of dopamine receptors by dopamine released centrally by amphetamine (Snyder, 1972). Using these criteria it has been shown that several chlorpromazine metabolites have dopamine receptor blocking activity. Thus, several of these metabolites increase dopamine turnover (Nyback & Sedvall, 1972), increase homovanillic acid accumulation in the striatum (Dailey, Sedvall & Sjoqvist, 1972) and antagonize amphetamine-induced stereotypy in rats (Lal & Sourkes, 1972).

Dopamine has been shown to produce an increase in cyclic AMP production by homogenates of the rat neo-striatum and in bovine retina, and this effect is blocked by chlorpromazine (Kebabian, Petzold & Greengard, 1972; Brown & Makman, 1973). It has been suggested that this system may constitute an *in vitro* model for cns dop-

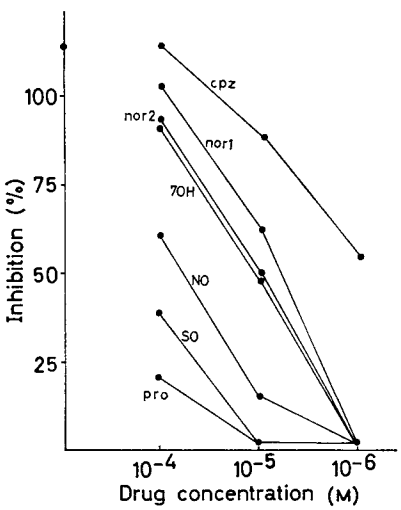


FIG. 1. The graph shows the percent inhibition produced by drugs at various concentrations of the rise in cyclic AMP production produced by 100 $\mu$M dopamine in rat striatal homogenates. Basal rates of production of cyclic AMP were 34·4 $\pm$ 3·2 p mol per incubation tube and stimulated rates were 61·2 $\pm$ 9·7 p mol. Points represent mean values for between 6 and 12 separate incubations. Standard errors $<10\%$. At concentrations of $10^{-4}$M the chlorpromazine and desmethylchlorpromazine produced inhibition of basal cyclic AMP production and this has been represented as inhibition of $>100\%$. For abbreviations see Table 1.

Table 1. *Concentration of drugs causing 50% of inhibition of the increase in cyclic AMP accumulation caused by 100 μM dopamine in rat striatal homogenates.*

| Drug | IC50 M | Maximum inhibition % |
|---|---|---|
| Chlorpromazine (cpz) | $1{\cdot}3 \times 10^{-6}$ | 115* |
| Desmethylchlorpromazine ($nor_1$) | $6 \times 10^{-6}$ | 105* |
| Bisdesmethylchlorpromazine ($nor_2$) | $1{\cdot}1 \times 10^{-5}$ | — |
| 7 Hydroxychlorpromazine (7-OH) | $1{\cdot}4 \times 10^{-5}$ | — |
| Chlorpromazine nitroxide (NO) | $5 \times 10^{-5}$ | — |
| Chlorpromazine sulphoxide (SO) | $>10^{-4}$ | — |
| Promethazine (pro) | $>10^{-4}$ | — |

* Drugs inhibited basal cyclic AMP levels as shown in Fig. 1.

amine receptors. We have, therefore, examined the effect of chlorpromazine and some of its major metabolites on the dopamine-stimulated increase in cyclic AMP production in rat striatal homogenates, using the procedure of Kebabian & others (1972). Male Wistar albino rats were decapitated and their brains removed in a cold room (4°). The neostriatum was removed and immediately homogenized in 25 vol of ice cold buffer containing 2 mM tris-(hydroxymethyl) aminomethane maleate (pH 7·4) and 2 mM EGTA. 50 μl aliquots of this homogenate were added to tubes containing 250 μl of ice cold buffer containing 80 mM tris-(hydroxymethyl)aminomethane maleate (pH 7·4), 2·0 mM $MgSO_2$, 10 mM theophylline hydrate and 0·2 mM EGTA. The reaction was initiated by addition of ATP to give a concentration of 0·5 mM. The tubes were incubated at 30° for 2·5 min in a shaking water bath. The reaction was terminated by placing the tubes in a boiling water bath for 4 min. The contents of each tube were centrifuged in a Beckman bench microfuge to remove insoluble material and a 25 μl aliquot of the supernatant was removed from each tube and dried down over phosphorus pentoxide in flat bottomed glass test tubes. Tubes were assayed for cyclic AMP content by the method of Gilman (1970).

The effect of drugs at various concentrations on the stimulation of cyclic AMP production by 100 μM dopamine is shown in Fig. 1. Drug concentrations causing 50% inhibition of the effect of 100 μM dopamine are shown in Table 1. The results show that the mono- and bis-desmethyl derivatives of chlorpromazine and to a lesser extent 7-hydroxy chlorpromazine blocked the effect of dopamine on cyclic AMP levels but were less potent than chlorpromazine. The results support the findings of Nyback & Sedvall (1972) that these metabolites increased dopamine turnover in mouse brain, and of Dailey, Sedvall & Sjoqvist (1972) who found that they increased homovanillic acid concentrations in mouse brain. Chlorpromazine nitroxide was not very effective in antagonizing the effect of dopamine, although it was able to increase dopamine turnover and homovanillic acid accumulation appreciably in the *in vivo* studies cited. It has been shown, however, that chlorpromazine nitroxide can be reduced to chlorpromazine *in vitro* by a liver microsomal preparation (Coccia & Westerfield, 1967), and it is therefore possible that the effects of this substance on dopamine metabolism observed *in vivo* are in fact due to its conversion to chlorpromazine. Chlorpromazine sulphoxide was inactive in our test system, and it is also ineffective in altering dopamine metabolism *in vivo*.

In conclusion it appears that several metabolites may play a direct role in chlorpromazine clinical/neuroleptic action. The 7-OH, $nor_1$ and $nor_2$ metabolites have been detected in the plasma of patients and may reach levels comparable with the parent drug (Curry & Marshall, 1968). It may therefore be important to monitor plasma levels of several chlorpromazine metabolites, as well as the parent compound to explain the lack of correlation between plasma levels of unchanged chlorpromazine and its therapeutic effect.

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# The absorption of aspirin and paracetamol in patients with achlorhydria

It is generally accepted that the site of absorption of weakly acidic and basic drugs in the gastrointestinal tract is determined by intraluminal pH. According to the pH-partition theory,weak organic acids are rapidly absorbed from the stomach (pH 1–2), and weak organic bases from the small intestine (pH 5·5–6·5). It follows that an increase in gastric pH should reduce the rate of absorption of acidic drugs from the stomach.

Acetylsalicylic acid is a moderately strong acid (pKa 3·5) and its gastric absorption should be pH dependent. Paracetamol is a much weaker acid (pKa 9·5) which is largely unionized at all pH values normally found in the gastrointestinal tract, and its absorption should not be appreciably influenced by changes in gastric pH. Acetylsalicylic acid and paracetamol absorption was therefore studied in achlorhydric and control patients. The groups were comparable for age, weight and sex. [Controls: 3 females, 3 males, mean wt (kg $\pm$ s.d.) 62·5 $\pm$ 13·3, mean age (years $\pm$ s.d.) 56·2 $\pm$ 10·2; achlorhydric patients: 3 females, 3 males, mean wt (kg $\pm$ s.d.) 69·8 $\pm$ 15·2, mean age (years $\pm$ s.d.) 65·2 $\pm$ 4·1]. After an overnight fast, each patient was given 1·5 g paracetamol (Panadol-Bayer) with 50 ml water, and serial plasma samples were obtained for 8 h. Two days later, the same patients were given 900 mg of acetylsalicylic acid (aspirin-Boots) under the same conditions, and samples were taken as before. The subjects were not permitted to walk, eat, drink or smoke for 2·5 h after taking the drugs, nor were they given any other medication on the day of the experiment. The achlorhydric patients had been shown to produce no gastric acid after pentagastrin stimulation (6 $\mu$g kg$^{-1}$); the controls were convalescent with no evidence of diseases associated with achlorhydria. Plasma concentrations of paracetamol were estimated