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Melanocyte-stimulating hormone—mimetic action of the phenothiazines

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We have compared the melanophore-stimulating action of four phenothiazines, trifluoperazine, perphenazine, chlorpromazine, and prochlorperazine, with α-MSH on the skin of the lizard *Anolis carolinensis*, using a new rate method of bioassay. The dose-response curves for the phenothiazines were parallel to that of α-MSH, and when given together α-MSH and chlorpromazine were additive. The phenothiazines may therefore stimulate melanosome dispersion in the lizard skin by the same mechanism as α-MSH; a MSH-mimetic action of phenothiazines may similarly explain their pigmentary action in man. The pigmentary potency of the phenothiazines corresponded with their therapeutic potency in man; this is in keeping with a neuro-regulatory role for MSH peptides and suggests a possible therapeutic use for them.

Pigmentation occurs in patients on prolonged phenothiazine treatment and is associated with cutaneous melanization (Blois, 1965; Satanove, 1965). Phenothiazines block release of hypothalamic inhibitory factors which are controlled by dopamine. Thus, in the rat, phenothiazines increase pituitary melanocyte-stimulating hormone (MSH) secretion (Kastin & Schally, 1966; Thody, Penny & others, 1975; Penny & Thody, 1976). Similarly, in man, phenothiazines cause increased plasma prolactin (Turkington, 1972), but it has recently been demonstrated that neither plasma immunoreactive β-MSH (Plummer, Thody & others, 1975; Smith, Goolamali & others, 1977) nor α-MSH (Clark, Smith, Thody & Shuster, in preparation) are increased by these drugs in man. We therefore wondered whether the phenothiazines might stimulate melanogenesis directly as do the MSH peptides (Lerner & McGuire, 1961). Since the action of MSH peptides on melanogenesis in the mammalian melanocyte parallels their action on melanosome dispersion in the melanophores of lower vertebrates, we have compared the action of the phenothiazines and α-MSH on the melanophores of the lizard skin using a new bioassay method based on rate of pigmentation change (Carter & Shuster, 1978).

METHODS AND MATERIALS

Standard and phenothiazines. Synthetic α-MSH was obtained from Ciba-Geigy Ltd and the following phenothiazines were assayed from the materials used therapeutically as chlorpromazine (Largactil), perphenazine (Fentazin), prochlorperazine (Stemetil), and trifluoperazine (Stelazine).

Lizards. The lizards (*Anolis carolinensis*) were obtained from De Natuurvriend, Donkeregaard, Utrecht, and were housed in a terrarium with 14 h light and 10 h dark. A temperature gradient of 20°-35° was maintained across the terrarium during the light hours while the minimal temperature attained at night was 18°. The lizards were fed with Tenebrio flies and larvae, and the terrarium was sprayed twice daily with water which the animals drank by licking the droplets off the vegetation.

Bioassy. The rate method has been reported elsewhere (Carter & Shuster, 1978). When green skin fragments are incubated in doubling dilutions of buffered solutions of α-MSH, their colour changes to brown and the time for colour change is recorded visually. The relation between the reciprocal of darkening time, the darkening speed, and logarithm of dose is sigmoid, and the logarithm of this response against logarithm of the dose is linear over the lower dose-response range. The ED50 for the original data is coincident with this dose range and is used in the assay. This method was used to measure the dose-response curves for α-MSH and the phenothazines, each assay being carried out in duplicate. In a separate experiment, the response of the melanophores to doses of α-MSH, chlorpromazine, and α-MSH + chlorpromazine was studied.

Statistical treatment of the data was by analysis of variance (Bliss, 1951).

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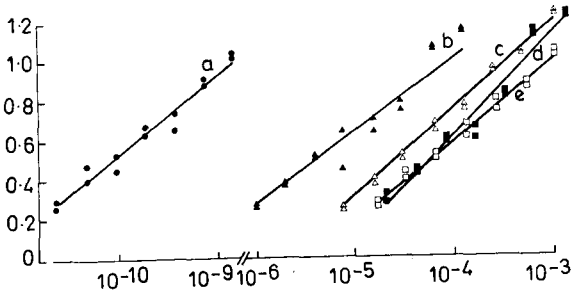

FIG. 1. Dose-response curves for a: α-MSH, b: trifluoperazine, c: perphenazine, d: chlorpromazine, and e: prochlorperazine. Each dose-response curve was highly significant ($P < 0.01$) and only the chlorpromazine dose-response curve deviated significantly from that of the standard, α-MSH ($P < 0.01$). Ordinate: Log darkening speed ($1/(s \times 10^3)$). Abscissa: Dose (M).

Table 1. *Potency of phenothiazines relative to α-MSH and to trifluoperazine calculated on a molar basis from the dose-response data of Fig.* 1·95% fiducial limits of the estimated potency (Bliss, 1951) are given in parenthesis.

Drug	Relative to α-MSH	Relative to trifluoperazine
α-MSH	1·00	71994
Trifluoperazine	$13{\cdot}89 \times 10^{-6}$ ($11{\cdot}92 \times 10^{-6}$ & $16{\cdot}20 \times 10^{-6}$)	1·00
Perphenazine	$3{\cdot}098 \times 10^{-6}$ ($2{\cdot}939 \times 10^{-6}$) & $3{\cdot}266 \times 10^{-6}$)	0·223
Chlorpromazine	$1{\cdot}765 \times 10^{-6}$ ($1{\cdot}587 \times 10^{-6}$ & $1{\cdot}964 \times 10^{-6}$)	0·127
Prochlorperazine	$1{\cdot}392 \times 10^{-6}$ ($1{\cdot}281 \times 10^{-6}$ & $1{\cdot}511 \times 10^{-6}$)	0·100

RESULTS

The pigmentary response to α-MSH and the four phenothiazines was linear in the range studied ($P < 0.01$, Figs 1 and 2). The mean index of precision with s.d. (Bliss, 1951) was 0·106 (0·028). In one assay the slope of the chlorpromazine dose-response deviated slightly, but significantly from that of α-MSH ($P < 0.01$, Fig. 1), but in the second it did not ($P < 0.05$, Fig. 2). The dose-response curves for the other phenothiazines did not deviate significantly from the slope of the standard ($P > 0.05$).

The potency of the phenothiazines was calculated relative to α-MSH and to trifluoperazine (Table 1). The minimal detectable dose of chlorpromazine was 22 μM (8·1 μg ml^{-1}).

Fig. 2 shows superimposed the dose-response curves for α-MSH and chlorpromazine, together with the dose-response curve for the combined solution of α-MSH + chlorpromazine. The potency ratio histogram in Fig. 2 shows the potency of the superimposed dose-response of chlorpromazine and that of the α-MSH + chlorpromazine relative to α-MSH. The sum of the potencies of the two individual dose-response curves is equal to that of the combined solution dose response curve, and therefore α-MSH and chlorpromazine are additive.

DISCUSSION

The present findings are that the four phenothiazine drugs have a direct pigmentary action on the reptilian melanophore. The parallel dose-response

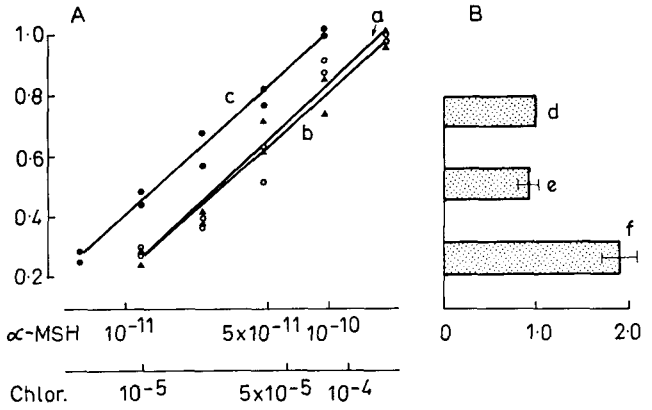

FIG. 2. A. Superimposed dose-response curves for a: α-MSH, b: chlorpromazine and c: α-MSH + chlorpromazine. Each dose-response curve is highly significant ($P < 0.01$), and those of chlorpromazine and α-MSH + chlorpromazine did not deviate significantly from that of α-MSH ($P > 0.05$). B. The potency ratio histogram indicates the potency of the chlorpromazine (e) and α-MSH + chlorpromazine (f) dose-response curves relative to that of α-MSH (d); the sum of the potencies of α-MSH and chlorpromazine is equal to the potency of the combined solution, α-MSH+ chlorpromazine. 95% fiducial limits of the estimated potencies are demonstrated as horizontal bars. A. Ordinate: Log darkening speed ($1/(s \times 10^3)$). Abscissa: Dose (M). Chlor.: chlorpromazine.

of α-MSH and the phenothiazines and the additive rather than synergistic effect of α-MSH + chlorpromazine suggests that the phenothiazines act in the same way as α-MSH on the reptilian melanophore, possibly through the same receptor. Since the MSH peptides which cause melanogenesis in human (Lerner & McGuire, 1961) and other mammalian melanocytes (Snell, 1964) also induce reptilian melanosome dispersion, it may be that the phenothiazines have a similar MSH-like action in man. Pigmentation in patients treated with chlorpromazine has usually been associated with prolonged high doses of the drug (e.g. Satanove, 1965) and plasma chlorpromazine concentrations have been reported to be as high as $1\,\mu g\,ml^{-1}$ (Rivera-Calimlin, Castenula & Lasagne, 1973).

Although the minimal concentration of chlorpromazine required to pigment lizard skin was found to be $22\,\mu M$ ($8{\cdot}1\,\mu g\,ml^{-1}$) these concentrations are likely to be achieved in the human melanocyte which appears to concentrate the drug (Satanove, 1965). Thus the pigmentation associated with phenothiazine administration in man appears not to be due to increased MSH release (Plummer & others, 1975; Smith & others, 1977) but may be due to an MSH-like action of these drugs on the melanocyte itself. Direct confirmation of this suggestion must await a mammalian melanocyte bioassay.

It is interesting that melanophores should respond to neuroleptic drugs, such as the phenothiazines, since both neural tissue and the melanophores are derived from the neural crest. Indeed, the order of potency of the phenothiazines which we have found is the same as the order in which they block the hypothalmic inhibitory action of dopamine on pituitary MSH secretion in the rat (Kastin & Schally, 1966) and in the frog (Scott & Nading, 1961). Furthermore, the order is the same as that in which they block the dopamine sensitive-adenylate cyclase system in the rat brain (Miller, Horn & Iversen, 1974) and this correlates with clinical effectiveness. Thus, the assay we have used might well prove to be a useful method for screening neuroleptic agents such as the phenothiazines.

Preliminary observations in our laboratory indicate the presence in human brain of high concentrations of immunoreactive α-, β- and bioactive MSH; this and the high concentrations of immunoreactive β-MSH in the csf (Smith & Shuster, 1976) suggests a neuroregulatory role for MSH peptides in man (Shuster, Smith & others, 1977; Shuster, Carter, Thody, Smith, Fisher & Cook to be published). The similar pigmentary action of the neuroleptic phenothiazines and α-MSH is in keeping with that view and suggests the need explore the therapeutic potential of MSH peptides as neuroleptic drugs.

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