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Relative Effectiveness of Phenothiazine Tranquilizing Drugs Causing Release of MSH.*† (26246)

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The ataraxic drug meprobamate (Miltown, Equanil) has been found to cause darkening of the light-adapted frog. This reaction does not occur in the hypophysectomized animal (1). Chlorpromazine (Thorazine), methoxypromazine (Tentone), and reserpine also have been found to cause melanophore dispersion in the normal frog and in the sand dab, *Lophosetta maculata.* On the other hand, azacyclonol (Frenquel) and oxanamide (Quiactin) have been found to be ineffective (2).

This paper is a report of the influence of 10 phenothiazine derivatives now used in medical practice on the pituitary melanocyte system of the frog, *Rana pipiens.* Marked differences in milligram potency of the drugs causing release of melanocyte stimulating hormone (MSH) from the hypophysis were observed.

Methods. Three hundred and thirty-one medium-sized *Rana pipiens* of both sexes were used in determination of the minimal effective doses (MED) of the drugs. The effects on 10 hypophysectomized frogs were also observed. Frogs were weighed and placed singly in 1000 ml beakers containing 200 ml of tap water. To provide a white background, beakers were wrapped with white cloth. Beakers containing the frogs were placed in white enamel pans under continual illumination from a pair of 4 foot, 40 watt fluorescent tubes mounted in a fixture 20 inches above the pans. Frogs were permitted to light-adapt overnight prior to injection. Equal mg% doses (mg of drug per 100 g of frog) of a drug were injected (0.1-1.3 ml) intraperitoneally into a series of 3 to 5 light-adapted frogs. Doses ranged from 0.01 to 8.0 mg%. The extent of melanophore dispersion was recorded from microscopic observation of the web of the foot at 2-5 hour intervals for 36 hours following injection. The melanophore index of Hogben and Slome(3) was used. Twenty-seven control frogs injected with carrier solutions and 5 uninjected controls were also run.

The mean melanophore response to each dose was calculated by averaging the single maximal responses of all frogs(3-11) injected with that dose. The MED was defined as the minimal dose eliciting a mean melanophore response of 3.0 on the Hogben and Slome index and was obtained by interpolating from a dose-response curve.

Hypophysectomy was performed according to the procedure of Hogben(4) with additional use of an ice bath to reduce blood flow. Complete removal of the hypophysis

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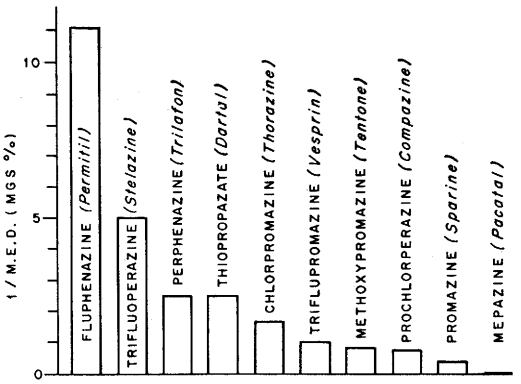

FIG. 1. Potency of phenothiazine tranquilizing drugs in causing activation of melanophores by MSH.

was indicated by punctate melanophores after one to 3 days on an illuminated, black cloth background following the operation.

The following drugs were used: methoxypromazine (Tentone), chlorpromazine (Thorazine), triflupromazine (Vesprin), trifluoperazine (Stelazine), prochlorperazine (Compazine), thiopropazate (Dartal), fluphenazine (Permitil), promazine (Sparine), mepazine (Pacatal), and perphenazine (Trilafon). Because of the relative insolubility of Trilafon and Pacatal, these drugs were suspended in an amphibian Ringer's solution containing 25% ethanol. All other drugs were dissolved in amphibian Ringer's.

Results and discussion. The reciprocal of the MED was used to compare the relative effectiveness of the various drugs (Fig. 1). All drugs caused melanophore dispersion in normal frogs with the exception of Pacatal. Carrier solutions did not cause darkening.

All drugs tested in hypophysectomized animals were without effect. These included Permitil, Stelazine, Dartal, Thorazine, Tentone and the non phenothiazine ataraxics meprobamate and reserpine.

Since the ataraxic drugs employed cause no dispersion of melanophore pigment in hypophysectomized frogs, it is concluded that these drugs bring about release of MSH by direct action on the pituitary gland or indirectly *via* brain centers. The possibility is recognized that the effect may be one of potentiation of MSH instead of release of active hormone. Experimental evidence indicates this not to be the case.

Permitil (1 mg%) does not delay paling when injected into frogs shortly after hypophysectomy while circulating MSH is presumably still present; nor did Permitil produce darkening in animals injected with subthreshold doses of Pituitrin (Parke Davis).

There is evidence reported in the literature that the release of certain pituitary hormones is chronically inhibited by the nervous system and that drugs may effect their release by counteracting this inhibition. It was suggested several years ago(5) that melanophore hormone might be under inhibitory hypothalamic control in the tadpole.

The data (Fig. 1) are of interest in relation to the chemical structures of the phenothiazine drugs (Fig. 2). The classes of drugs are distinguished on the basis of substitution of a piperazinylpropyl, a dimethylaminopropyl, or a piperidylmethyl group at position 10 of the phenothiazine nucleus. Drugs within these classes are characterized by further substitutions at position 2 of the phenothiazine nucleus and at position 1 of the piperazine ring.

Relationships between various substitutions on the phenothiazine nucleus and milligram potency for pigment dispersing action are summarized as follows: 1. The most active drugs (Permitil and Stelazine) contain in trifluoromethyl group and the piperazine ring. 2. The next 2 most active derivatives (Trilafon and Dartal) contain chlorine and the piperazine ring. 3. With the exception

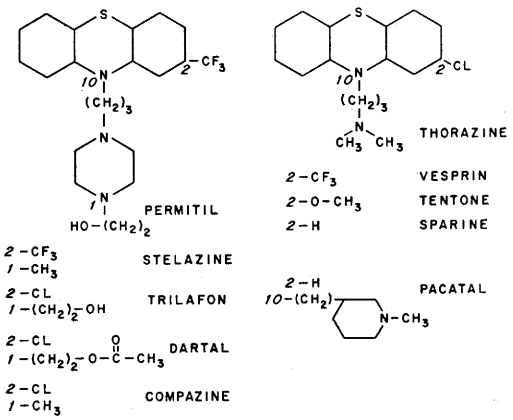

FIG. 2. Chemical structures of phenothiazine tranquilizing drugs. Substitutions are indicated at positions 1, 2 and 10.

of Compazine, the next 5 drugs, in decreasing order of milligram potency (Thorazine, Vesprin, Compazine, Tentone, and Sparine), contain the dimethylamino group in place of the piperazine ring. 4. The only ineffective drug, (Pacatal) is the only one containing the piperidine ring.

It can therefore be concluded that of the 3 substituents at position 10, the piperazinylpropyl group is more effective than the dimethylaminopropyl group and that the piperidylmethyl group produces an ineffective drug. Trifluoromethyl is generally more effective than chlorine or other substituents at position two.

Our results show a striking parallel with the results reported by Ayd(6) comparing the milligram potency of these same drugs required for therapeutic control of a wide spectrum of psychiatric disorders in 4,000 ambulatory and hospital patients. The relative ranking of the 10 drugs for milligram potency was identical in both studies with the exception of Thorazine, which was less effective than Compazine and Vesprin in Ayd's study, and of Compazine, which Ayd found to be more effective than Vesprin and Tentone.

It may be concluded that the relative milligram potencies of phenothiazines are similar for pigment dispersion in the frog melanocyte and for therapeutic control of human psychiatric illness.

Summary. Nine phenothiazine ataraxic drugs were found to cause melanophore dispersion in the intact frog; one drug was shown to be ineffective. All drugs tested in hypophysectomized animals were without effect. The drugs, therefore, are effecting the release of MSH by direct action on the hypophysis or indirectly *via* brain centers. Minimal effective doses of the drugs were correlated with substitutions on the phenothiazine nucleus. A parallel with milligram potency for therapeutic control of psychiatric disorders is noted.

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Direct Recordings from Chronically Implanted Electrodes on the Specialized Conducting System of the Heart.* (26247)

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This laboratory has previously described a technic for studying conduction within the heart by utilizing electrodes placed at selected sites on the endocardium under direct vision during cardiopulmonary bypass(1,2, 3). Such studies, until recently, have been carried out only during acute experiments. It is now possible to implant multi-lead electrodes over the bundle of His, the Purkinje-papillary junctions and on other areas within the heart with complete recovery of the animal from the surgical procedure. Records from these intracardiac electrodes, as well as from surface electrodes on the atria and ventricles, can be monitored through external leads over extended periods of time.

Method. Adult mongrel dogs weighing 20-28 kg were anesthetized with an intravenous injection of a 2½% solution of thiopental sodium, intubated, and placed on a respirator.

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