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RELEVANCE OF THE N,N-DIMETHYL CONFIGURATION TO THE PHARMACOLOGICAL ACTION OF CHLORPROMAZINE

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Abstract—The pharmacological effectiveness of chlorpromazine, desmonomethyl chlorpromazine, and desdimethyl chlorpromazine was determined by relating drug-induced depression of reactivity to phenothiazine levels in rat brain. In addition, the relative potency of these drugs in raising thresholds for maximal electroencephalographic (EEG) recruitment and blocking of EEG-alerting response to pain was investigated in rabbits. The results indicate that progressive demethylation of chlorpromazine is associated with an increasing loss of its pharmacological potency. Conversely, toxicity was found to be highest for desdimethyl chlorpromazine followed by desmonomethyl chlorpromazine and chlorpromazine in decreasing order. It is concluded that the N,N-dimethyl configuration plays a major role in the pharmacological effectiveness of chlorpromazine.

WITH the introduction of chlorpromazine into the treatment of hyperactive psychotic patients, various phenothiazine derivatives have been synthesized in order to find more potent and more specifically acting drugs and, at the same time, to learn more about the physiology and pathophysiology of brain function. This report is concerned with the significance of the N,N-dimethyl configuration of chlorpromazine in the pharmacological action of this drug. This question was approached by determining the effects of chlorpromazine (tertiary amine), desmonomethyl chlorpromazine (secondary amine), and desdimethyl chlorpromazine (primary amine) on overt behavior in relation to the phenothiazine levels in the brain of rats. In addition, the effects of these chemical agents on thalamocortical recruitment and EEG alerting were determined in rabbits.

METHOD

I. Combined behavioral and chemical studies in rats

A total of 81 male albino rats of the Wistar stock, weighing from 300 to 400 g, were used in this part of the investigation, which consisted of a series of individual experiments. For each experiment the rats were divided into four groups. One group of two animals received placebo, and each of the other three groups, consisting of four to six rats each, was given one of the three drugs. All compounds were dissolved in polyethylene glycol 400 shortly before administration. Equimolar dosage levels for the three drugs, ranging from 0·0422 to 0·1266 mmole/kg, were administered by intraperitoneal injection in the individual experiments. All drugs are reported as the

respective hydrochlorides. Behavior was evaluated shortly before injection and at certain time intervals, usually 90 min, after administration of the drug. Immediately after completion of the behavioral observations, the animals were sacrificed by decapitation and phenothiazine concentrations in the brain were determined. The following methods were employed.

Behavior. Behavior was estimated by evaluating spontaneous activity, intensity of the righting reflex, and intensity of the responses to various external stimuli including pain, touch, air blowing, inhalation of chemicals (vanillin and ammonia), and noise. Reduction in the intensity of these reactions was termed "depression of reactivity" and was rated individually by the independent judgments of two of the authors (EEB and HHK) according to four degrees of severity: 0, no depression of reactivity; 1, mild depression; 2, moderate; and 3, marked. This four-step scale permitted sufficient latitude of judgment to describe the changes in behavior encountered without, at the same time, forcing the judges to make discriminations beyond their powers of observation. Since both observers knew what was being administered, the reliability of the procedures and of the observers' ability to discriminate was checked at intervals on a blind basis with full randomization of animals and procedures. Differences between the individual ratings of the two judges were never in excess of one unit of behavioral reactivity and, consequently, the individual ratings of the investigators were pooled for each behavioral parameter, and the average score of all parameters was then used to designate depression of reactivity. The final assessment of drug action was calculated by subtracting the average rating obtained before drug administration from that obtained after injection of the drug.

Brain phenothiazine concentrations. Brain phenothiazine concentrations were determined spectrophotometrically. The mode of extraction was similar to the procedure described by Salzman and Brodie[1] except that the heptane extract was not washed with acetate buffer because marked quantities of desmonomethyl and desdimethyl chlorpromazine were lost by that process. By omitting washing, however, phenothiazine sulfoxide was also taken through the procedure, and therefore a method of quantitation had to be employed which allowed the concentrations of both phenothiazines and sulfoxides to be calculated. For this purpose a pair of simultaneous equations was used, relating to optical densities at the wave lengths of 236 and 265 mμ to the sums of the products of the extinction coefficients of each compound, multiplied by its concentration. Solution of these equations yielded the following expressions representing the basis for the calculation of the concentrations of phenothiazines and sulfoxides:

$$\text{(C. phenothiazine)} = \frac{\text{O.D. 236 m}\mu - 3{\cdot}95\,(\text{O.D. 265 m}\mu)}{-0{\cdot}063} \quad \text{and}$$

$$\text{(C. sulfoxide)} = \frac{\text{O.D. 236 m}\mu - 1{\cdot}79\,(\text{O.D. 265 m}\mu)}{+0{\cdot}048}$$

These concentrations are expressed in terms of micrograms per milliliter of solution. As only very small amounts of phenothiazine sulfoxides were detected in the brain after injection of chlorpromazine or its demethylated derivatives, it should be expected that the phenothiazine concentrations calculated from the equation above should

correspond closely to the values derived from the standard curve reported by Salzman and Brodie,[1] and this correlation was found. The spectrophotometric curves for authentic chlorpromazine, desmonomethyl, and desdimethylchlorpromazine were found to be identical; the curves of the final brain extracts were identical with those of the authentic compounds. Slight deviations, when they occurred, reflected the presence of phenothiazine sulfoxide. Incubation of 0·28 and 0·56 μmole of chlorpromazine and its demethylated derivatives with brain homogenates yielded an average recovery of 73% for each of the three compounds by the method employed.

II. Electrophysiological studies

Changes in the gross activity of the central nervous system were determined from the EEG patterns of the rabbit. Assessments were based on threshold measurements of the recruitment responses of the nonspecific thalamocortical projection system and also on the depression of EEG alerting produced by peripheral stimulation.

Thirty New Zealand male albino rabbits, weighing approximately 3 kg, were employed in experiments with chlorpromazine and its demethylated derivatives. Animals were tracheotomized under ether and local pontocaine anesthesia, curarized, and artificially respired prior to the administration of drugs.[2] Each compound was dissolved in distilled water and administered by femoral vein.

Thalamocortical recruitment. The electrodes for thalamic stimulation were placed in the approximate region of the nucleus centrum medianum according to the charts of Sawyer[3] for the rabbit. Coordinates were as follows: posterior 5 mm, lateral 2 mm, and vertical −1 mm. The final location of the stimulating electrodes was chosen according to functional criteria with the final electrode depth adjusted for maximal recruitment at the least current voltage. An animal was not utilized for experimentation if more than 7 V of stimulating current was required to produce recruitment prior to the administration of the drug. The stimulating electrodes consisted of a pair of Formvar-coated nichrome wires twisted together. The tips were cut in a slanted plane with a distance between bare surfaces not in excess of 1 mm. This gave a parallel bipolar electrode configuration for stimulation. Cortical electrodes of the silver-ball type were placed symmetrically in the anterior cortex at positions 4 mm and 8 mm from the bregma. Stimulation was delivered by means of a Grass model S-4 stimulator and model SIU-4B stimulus isolation unit. The stimulating current consisted of 0·3-msec square waves at a frequency of 12 cycles/sec. No attempt was made to monitor pulse current. Voltage measurements were expressed in terms of changes in the voltage-dial setting on the stimulation apparatus.

Recruitment threshold was defined as the minimal voltage necessary to produce a continuous recruiting response throughout a 10-sec period of stimulation. This is a high intensity recruitment threshold, where recruiting begins at the second or third repeating stimulus, reaches maximal amplitude and continues without variation until the termination of the stimulus. Little waxing and waning is apparent at the voltage required to produce this maximal response, in contrast to recruitment patterns elicited at lower current intensities. After the initial voltage threshold had been determined, an initial drug dose of 0·0028 mmole/kg was given, followed by 0·0056 mmole/kg every 20 min to a total dose of 0·0197 mmole/kg. The voltage required to elicit the desired recruitment response was determined at an interval of 10 min after each injection and at 90 min after the last injection of drug.

EEG-alerting response. Each drug was also studied in five rabbits for block of the EEG alerting produced by peripherial stimulation. The compounds were administered by femoral vein at the rate of 0·0028 mmole/kg every 4 min until the animals succumbed. Monopolar recordings were obtained from the anterior and posterior cortex, caudate nucleus, thalamus, hippocampus, amygdala, and reticular substance just cephalad to the pons. Histological examinations were not conducted since no attempt was made to localize drug effects to a particular brain structure. Animals were stimulated at the end of each 4-min period by applying pain in the form of a pinch to the hindpaw. Block of EEG alerting was considered to be that point in the serial drug administration when pain failed to elicit a change in the EEG pattern either cortically or from all areas recorded.

RESULTS

Figure 1 relates drug-induced depression of reactivity to the phenothiazine concentrations in the rat brain observed after injection of chlorpromazine, desmonomethyl

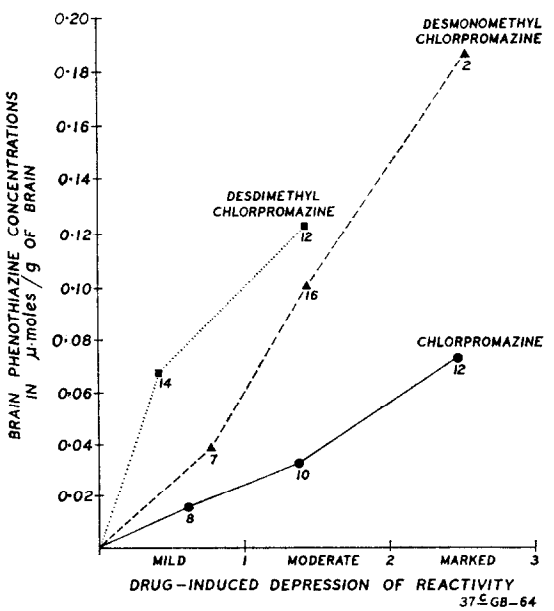

FIG. 1. Relationship between brain phenothiazine concentrations and drug-induced depression of reactivity in rats. For explanation see text.

chlorpromazine, and desdimethyl chlorpromazine. This figure shows that with all three drugs, the degree of depression of reactivity increased with rising phenothiazine concentrations in the brain. The circles designate the average concentrations for the number of animals indicated. It is evident from Fig. 1 that in terms of brain phenothiazine concentrations, chlorpromazine was about twice to three times as potent in depressing behavioral reactivity as desmonomethyl chlorpromazine. Desmonomethyl chlorpromazine, in turn, was more effective than the desdimethylated compound. A number of animals receiving desdimethyl chlorpromazine showed no degree of

depression of reactivity, and in several instances the converse effect of excitation was noted. When brain phenothiazine concentrations were brought to a level of about 0·08 μmole/g of brain tissue with a dosage of 0·1206 mmole/kg of desdimethyl chlorpromazine, no further transquilization was observed. Intsead, toxic effects including tremors and tonic–clonic convulsions were noted. Death usually occurred at brain phenothiazine concentrations of from 0·12 to 0·15 μmole/g of brain tissue in animals receiving desdimethyl chlorpromazine. The relatively high toxicity of this desdimethylated compound is apparent in Table 1. All four animals receiving chlorpromazine

TABLE 1. RATE OF SURVIVAL AFTER INTRAPERITONEAL INJECTION OF VARIOUS DOSES OF CHLORPROMAZINE AND ITS DEMETHYLATED DERIVATIVES IN RATS

Drug	Dose (mmole/kg)	No. of animals injected	No. of animals survived	No. of animals dead
Chlorpromazine	0·0422	5	5	0
	0·0844	5	5	0
	0·1266	4	4	0
Desmonomethyl chlorpromazine	0·0422	5	5	0
	0·0844	5	5	0
	0·1266	5	3	2
Desdimethyl chlorpromazine	0·0422	5	5	0
	0·0844	5	5	0
	0·1266	7	1	6

survived at a dose level of 0·1266 mmole/kg while two of the five rats receiving desmonomethyl chlorpromazine and six of seven rats receiving desdimethyl chlorpromazine at this dosage died within 24 hr. At lower doses of from 0·0422 to 0·844 mmole/kg, all animals survived regardless of the compound. Thus, a reciprocal relationship exists between toxicity and pharmacological activity for these three pharmacological agents.

The results of the electrophysiological studies parallel those obtained by evaluation of the degree of drug-induced depression of behavioral reactivity. Fig. 2 presents the

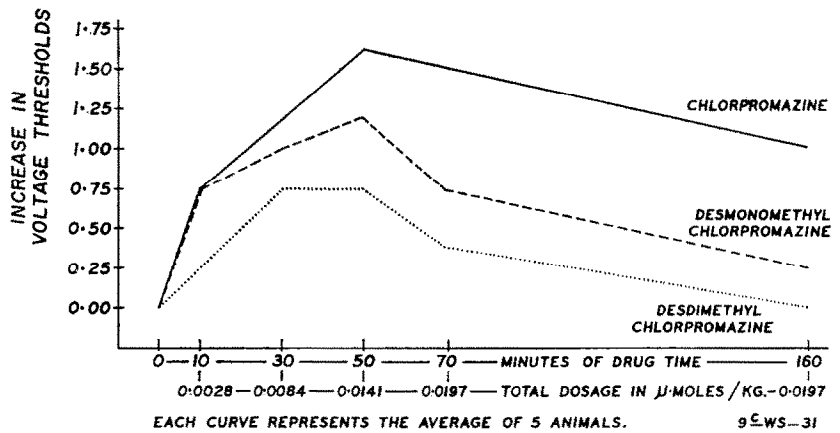

FIG. 2. Changes in threshold for maximal recruitment after intravenous injection of chlorpromazine and its desmethylated derivatives in rabbits. Each curve represents the average of 5 animals.

findings for changes in recruitment thresholds. Chlorpromazine had the greatest effect in elevating thresholds, followed by desmonomethyl chlorpromazine and desdimethyl chlorpromazine in decreasing order. The results on blockade of EEG alerting are presented in Table 2. Chlorpromazine was found to be the most potent in depressing EEG alerting to pain while desdimethyl chlorpromazine was found to be the least effective, leaving desmonomethyl chlorpromazine again in an intermediate position.

TABLE 2. BLOCK OF EEG-ALERTING RESPONSE TO PAIN AFTER INTRAVENOUS INJECTION OF CHLORPROMAZINE OR ITS DEMETHYLATED DERIVATIVES IN RABBITS

	Block of EEG alerting	
	Cortical (mmole/kg)	All areas recorded (mmole/kg)
Chlorpromazine	0·0079	0·0196
Desmonomethyl chlorpromazine	0·0146	0·0236
Desdimethyl chlorpromazine	0·0244	0·0329

Each drug was administered to 5 animals. The values for the drug doses represent averages of 5 experiments.

DISCUSSION

The metabolism of chlorpromazine yields a variety of products. Salzman *et al.*[4] identified chloropromazine sulfoxide as a major metabolite. Since then, many other metabolic products have been detected, and it has been estimated that about 24 chlorpromazine metabolites, including chlorpromazine sulfoxide, desmonomethyl chlorpromazine sulfoxide, desdimethyl chlorpromazine sulfoxide, and chlorpromazine N-oxide, are excreted into the urine.[5, 6] Subsequent studies conducted to determine the pharmacological potency of the metabolic products of chlorpromazine revealed that none of the metabolites so far tested was so potent as the parent compound. Chlorpromazine sulfoxide was markedly weaker than chlorpromazine in various species.[7, 8] Only traces of phenothiazine sulfoxide were extracted from the brain after administration of chlorpromazine or its demethylated derivatives in the present study. Thus, the metabolically formed phenothiazine sulfoxides do not exert any appreciable influence on the pharmacological actions of the three parent substances. Recently, Posner *et al.*[9] reported their results on the pharmacological effectiveness of some chlorpromazine and promazine derivatives. Based upon potentiation of hexobarbital sleeping time, stimulated locomotor activity, and conditioned responses controlled by reward and punishment, these authors concluded that phenothiazine sulfoxide was least active, whereas chlorpromazine-N-oxide, although more active, was less effective than either desmonomethyl chlorpromazine or chlorpromazine. Desmonomethyl chlorpromazine was found to be only slightly less active than chlorpromazine.

The purpose of the present study was to determine the significance of the N,N-dimethyl configuration in the pharmacological action of chlorpromazine. Consistent and marked differences in the pharmacological effectiveness of chlorpromazine, desmonomethyl chlorpromazine, and desdimethyl chlorpromazine were revealed by the several measures employed. Chlorpromazine was found to be two to three times more potent than desmonomethyl chlorpromazine, based upon the brain phenothiazine

concentrations, in depressing behavioral reactivity; desdimethyl chlorpromazine was the least active of the three compounds studied. Similar trends occurred in the alteration of EEG recruitment thresholds after thalamic stimulation and for the blockade of the EEG-alerting response to pain. These electrophysiological experiments also indicated, however, that chlorpromazine and desmonomethyl chlorpromazine were about equally effective during the first 30 min after drug administration, as measured by the recruitment response threshold, and it was only after this initial interval that the greater potency of chlorpromazine became apparent. Similarly, behavioral observations of rats disclosed that during an initial period of up to 90 min after drug, the degree of depression of reactivity was about the same in many instances for either chlorpromazine- or desmonomethyl chlorpromazine-treated animals and that major differences between these compounds were not apparent until later. Moreover, with the low dosage of 0·0422 mmole/kg, the onset of depression of reactivity was generally even more rapid with the desmonomethyl derivative than with chlorpromazine. In some instances, then, desmonomethyl chlorpromazine acts more rapidly but never so potently as chlorpromazine in inducing depressant changes in central nervous system activity. From these findings it may be concluded that the N,N-dimethyl configuration plays a major role in the pharmacological effectiveness of chlorpromazine and that progressive demethylation of this drug is associated with decreasing potency of action.

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