Effects of Some Psychotropic Agents on Peripheral Nerve Conduction Rate

By JACK K. PRUETT* and BYRON B. WILLIAMS

Impulse conduction rates in isolated frog sciatic nerves were reduced significantly by immersion of the nerves in solutions of chlordiazepoxide, 3.4×10^{-3} μm./ml. and prochlorperazine, 5.5×10^{-3} μm./ml. Promazine at a concentration of 3.5×10^{-3} μm./ml. did not significantly alter conduction rates. For chlordiazepoxide the conduction rate reduction increased with increase in drug concentration.

Most of the studies of psychotropic agents center around their effects on the central nervous system. While it has been established that the major component of psychotropic effect involves one or more central sites, there are side effects produced by drugs in this group which are suggestive of peripheral action, *e.g.*, tingling of extremities and impaired association movements (1–4). Plexus anesthesia in frogs induced by chlorpromazine (5) is indicative of the capacity of such a drug to affect peripheral nerve function.

Krivoy (6) reported peripheral nerve effects of several analgetic agents and speculated concerning the relationship of such effects to drug action mechanism. Tasaki (7) has shown that a 1.2% urethan–ringer solution reduced the conduction rate of a large motor fiber approximately 30% after exposure of less than 5 min. Although it may be argued that transmission reduction of such a magnitude by a drug solution at such a concentration would hardly explain the usual pharmacological effects of urethan, it does seem likely that the effects of urethan in large doses might reflect to some extent this peripheral action. It seems reasonable that psychotropic drugs also in high doses might have some of their nervous system activity attributed in part to an effect on peripheral nerve function.

This project was designed to provide preliminary information on the possible effect of some commonly used psychotropic drugs on impulse conduction rate in peripheral nerves. The drugs chosen, chlordiazepoxide,[1] prochlorperazine,[2] and promazine[3] represent two different structural categories.

EXPERIMENTAL

The sciatic nerve of the green frog, *Rana pipiens*, was used in this investigation. The nerve was dissected from the spinal cord to a point near the termination of the peroneal nerve. Care was taken to retain the eighth and ninth roots of the sciatic nerve, and the tibial nerve was trimmed away at the bifurcation of peroneal and tibial nerves. The dissected nerve was maintained in a beaker of frog ringer solution at pH 7.2 for 1 hr. after which time a general state of stability was attained (7). The nerve was then suspended from silver electrodes mounted in the nerve chamber. The proximal end of the nerve trunk was placed on a pair of stimulating electrodes, and the distal end on a pair of recording electrodes. The stimulating electrodes were 53 mm. from the recording electrodes.

Biphasic stimuli were employed to initiate impulses. Stimuli were provided by a Harvard electronic stimulator, model 340, isolated from the nerve chamber by an isolation transformer. The stimulus intensity was 0.5 v. and of 800 μsec. duration. The voltage constituted a submaximal stimulus for A fibers. The recording and measurement of impulse conduction rates were accomplished by standard electrophysiological techniques essentially like those of Krivoy (6) and Stacy (9) with instrumentation differences as described below. An all-purpose conductor-coupled Mark III preamplifier and a d.c. amplifier, both manufactured by E and M Instrument Co., were used in conjunction with a Lavoie Laboratories TS-239 A/UP oscilloscope. A model 800 Polaroid camera with close-up lens was mounted on the oscilloscope to allow photographic recording of the traces. Type 47, 3000 speed Polaroid film was used to insure high-contrast prints. The sweep generator of the oscilloscope was synchronized with the stimulator. An impulse from the stimulator served to start the sweep at the same time the nerve received a stimulus. The nerve, suspended on the electrodes as described above, was stimulated, and the trace with the resulting action potential was recorded photographically by opening the shutter for the duration of the trace. Conduction time was measured from the end of the stimulus artifact to the apex of the action potential spike. Time markers superimposed on the trace eliminated need for separate time signals. Time signals appeared on all traces and were imposed every 100 μsec. along the trace as shown in Fig. 1.

Nerve conduction rates were determined for the treatment groups and one control group. The treatment groups included chlordiazepoxide, 3.4×10^{-3} μm./ml.; prochlorperazine, 5.5×10^{-3} μm./ml.; and promazine, 3.5×10^{-3} μm./ml. Nerves from treatment groups were checked for normal conduction rate then immersed in drug-ringer solution for a period of 60 min., after which they were

Fig. 1.—Oscilloscope trace of action potential. Arrow indicates stimulus artifact.

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[1] Marketed as Librium Hydrochloride by Roche Laboratories, Nutley, N. J.

[2] Marketed as Compazine by Smith Kline & French Laboratories, Philadelphia, Pa.

[3] Marketed as Sparine by Wyeth Laboratories, Philadelphia, Pa.

TABLE I.—REDUCTION IN IMPULSE CONDUCTION RATE IN ISOLATED FROG SCIATIC NERVE

Treatment	Concn., μm./ml.	Variates, No.	Mean % Reduction	S.E.	*t* Test Probability
Control		18	1.04	1.25	
Chlordiazepoxide	3.4×10^{-3}	25	20.7	3.2	0.01
Prochlorperazine	5.5×10^{-3}	12	16.17	5.1	0.01
Promazine	3.5×10^{-3}	11	6.1	4	0.2

TABLE II.—VARIANCE ANALYSIS OF CHLORDIAZEPOXIDE CONDUCTION RATE EFFECT AT DIFFERENT CONCENTRATIONS

Component	Sum of Sq.	Degrees of Freedom	Variance	*F*	*F* at *P* = 0.01
Among groups	5158.91	3	1719.63	31.29	4.64
Within groups	1429.87	26	54.95		
Total	6588.78	29			

removed from the drug solution and a second rate recording made. Nerves in control groups were subjected to the same procedure except that no drug was added to the frog ringer solution. The chlordiazepoxide group comprised 25 nerves, the prochlorperazine group 12, the promazine group 11, and the control group 18.

To establish dose-effect relationship, four higher concentrations of chlordiazepoxide were used. Since some of these concentrations lowered the pH of the drug-ringer solutions, control nerves for these concentrations were treated with frog ringer solutions adjusted to the altered pH values. Drug treatment groups included from six to 12 nerves and all control groups included six nerves. The same general procedure was used as described above except that the period of immersion was 30 sec.

RESULTS

Single Concentrations.—Per cent change from normal conduction rate was determined for each treated nerve, and these data were subjected to statistical analysis. Table I presents data from tests in which single concentrations of each drug were used. Probability values indicate that the conduction rates were significantly lowered in comparison with controls by direct exposure of nerves to chlordiazepoxide and to prochlorperazine. Promazine treated nerves did not differ significantly from control nerves in conduction rate change at the drug concentration used.

Concentration-Effect Relationship.—Table II presents variance analysis data from tests in which four higher concentrations of chlordiazepoxide were used in an attempt to evaluate the concentration-effect pattern. Experimental *F* values were found to exceed table *F* values at 1% probability and, thus, indicated statistically significant difference between effects of the several drug concentrations. Comparison of control data at pH values corresponding to those of drug-ringer solutions revealed that the hydrogen-ion concentration of the solution used did not appreciably affect the conduction rate. Statistical analysis also indicated a significant difference between drug treated and control nerve conduction rates. Figure 2 graphically presents the relationship between concentration and per cent reduction in conduction rate. Data points as well as calculated regression points are shown. Regression analysis indicated no significant deviation from linearity at the 5% probability level.

DISCUSSION

Preliminary determinations of effects of three psychotropic agents on peripheral nerve impulse conduction rates revealed a significant rate reduction by chlordiazepoxide and prochlorperazine. Promazine at the concentration used failed to significantly alter the conduction rate. An investigation of the concentration-effect pattern for chlordiazepoxide indicated that within the limits of concentration used there was evidence of increase in rate reducing effect with increase in concentration of drug.

Although change in conduction rate of the magnitude revealed at these concentrations (10 to 30% reduction) would hardly be expected to account for the major pharmacological effects of these drugs, it would not be unreasonable to assume that there might be some contribution by such an axon effect to the total activity of these drugs at high dose levels. Evidence of this type may be considered of importance also as an indication of a capacity of some psychotropic drugs to exert general effects on nervous tissue in addition to their rather specific central nervous system effects. Such evidence may point also to a need for the consideration,

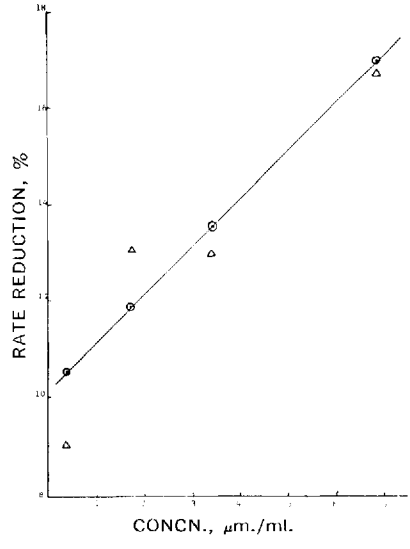

Fig. 2.—Relationship between conduction rate and concentration of chlordiazepoxide. Key: ⊙, calculated point; △, data point.

in mechanism studies, of the possibilities of conductional as well as junctional sites of action for psychotropic agents.

The need to determine site and mechanism of the conduction rate alteration is apparent. The drug concentrations used in this study were near the concentration of chlorpromazine found by Nathan and Friedman (8) to alter the permeability of resting cells of *Tetrahymena pyriformis*. They concluded that this alteration had a lipid site of action. Tasaki (7), in his experiments with saponin, has demonstrated that pharmacological alteration of lipid can change impulse conduction rate. He reported that this agent, by an effect on lipid of the myelin sheath, increased the time required for impulse transmission across the internodal segment. He found, however, that urethan altered conduction rate by a nodal rather than an internodal effect. A study of conduction parameters to provide information as to the site of the rate alteration by these psychotropic agents is planned.

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Ion-Exchange Separation and Ultraviolet Spectrophotometric Determination of Dextromethorphan in Pharmaceutical Products

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A method for the isolation and determination of dextromethorphan is presented. The dextromethorphan is extracted with strong cation exchange resin, AG 50W-X4, and is subsequently eluted with 1 *N* hydrochloric acid in 60 per cent methanol in water. The dextromethorphan is determined in the eluate by ultraviolet spectrophotometry. The assay is used successfully on several commercial products.

THE POPULAR acceptance of dextromethorphan as an antitussive agent has brought numerous pharmaceutical products to the consumer with this as the main ingredient. There has been very little information reported on the analytical procedures for the determination of dextromethorphan concentrations in liquid dosage forms.

Saques (1) was able to obtain satisfactory results using ultraviolet spectrophotometry and nonaqueous titration on the pure compound but control blanks had to be utilized to overcome interferences from excipients. Lee (2) demonstrated the use of the classical reineckate precipitation and colorimetric determination on the pure compound. Horioka (3) tested a dye complexation and extraction. The official method (4) for the assay of the pure compound and for the official tablets utilizes nonaqueous titration. Ultraviolet spectrophotometry and paper chromatography (5, 6) have also been studied.

The official assay procedure for the syrup requires an involved immiscible solvent extraction and reextraction followed by the determination using ultraviolet spectrophotometry.

The present paper utilizes the ability of strongly acidic cation exchange resins to separate an amine such as dextromethorphan from common dosage form ingredients prior to determination on a spectrophotometer. A weakly acidic solution is used prior to the use of the strongly acidic solution to remove traces of aromatic amines from flavors or coloring agents that may be present in pharmaceutical products. This type of separation using ion-exchange resins has been employed in the determination for phenylephrine by Kelly and Auerbach (7) and by Blake and Nona (8) in the determination for ephedrine salts.

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EXPERIMENTAL

Apparatus.—Glass column 20 cm. × 1 cm. with stopcock made of Teflon and containing built in needle valve for control of flow rate. The column is also fitted with a reservoir with a capacity of 250 ml.

A suitable recording ultraviolet spectrophotometer such as the Beckman DK-2A or Spectronic 505 which records in absorbance units.

Reagents.—Cationic exchange resin AG 50W-X4 100–200 mesh in hydrogen form available from Bio-Rad Laboratories, Richmond, Calif. Enough resin, about 3 Gm., is added in the form of a slurry to the glass column and rinsed with water. Hydrochloric acid, 0.05 *N* in 60% methanol in water. Hydrochloric acid, 1.0 *N* in 60% methanol in water.

Standard Solution.—Weigh exactly 150 mg. of dextromethorphan hydrobromide N.F. reference standard and transfer to a 100-ml. volumetric flask. Dissolve and adjust the volume with distilled water to prepare the desired stock standard.

Pipet exactly 10 ml. of the stock standard into a 200-ml. volumetric flask and adjust the volume with 1.0 *N* hydrochloric acid in 60% methanol in water.