of pregnancy and is needed to initiate uterine excitability and induce contractions at a force capable of expelling the fetuses.

It appears from this study that the administration of cyproterone acetate, an antiandrogenic, progestational compound, at midpregnancy prolongs the progesterone dominance. That the action of this compound is prolonged is in agreement with the findings of Bridge and Scott (2) who observed that prostatic secretion in dogs treated with cyproterone acetate was abolished for a period greater than 47 days after the termination of drug treatment.

The duration of action of this compound would also explain the increase in the gestational period of rats treated at midpregnancy with cyproterone acetate and the inability of these animals to deliver at term. Since the undelivered fetuses continue to grow, forceful and painful expulsion might account for the high percentage of cannibalization observed in these animals (4).

Since it was determined that the force of contractions is reduced following the administration of cyproterone acetate, investigations are presently in progress to assay the levels of estrogens and progesterone in the treated animals. The results obtained from these studies are important in elucidating the mechanism of action of this compound on uterine contractility.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 10, 1973, from the *College of Pharmacy, St. John's University, Jamaica, NY 11439*

Accepted for publication September 13, 1974.

Adapted in part from a thesis submitted by J. A. Catuogno to St. John's University in partial fulfillment of the Master of Science degree requirements.

[x] To whom inquiries should be directed.

Anticonvulsant Activity of *N,N'*-Bis[3-(3-substituted urea)propyl]piperazines

ARVIND K. CHATURVEDI [*], JAYANTI P. BARTHWAL [*], SURENDRA S. PARMAR [*‡x], and VIRGIL I. STENBERG [‡]

Abstract □ Several *N,N'*-bis[3-(3-substituted urea)propyl]piperazines were synthesized and characterized by their sharp melting points, elemental analyses, and IR spectra. All substituted piperazines were found to possess anticonvulsant activity, which was reflected by 20–70% protection observed against pentylenetetrazol-induced seizures in mice. Some of these compounds inhibited oxidation of pyruvic acid by rat brain homogenate. No correlation could be observed between the anticonvulsant activity possessed by these substituted piperazines and their ability to inhibit the oxidation of pyruvic acid.

Keyphrases □ *N,N'*-Bis[3-(3-substituted urea)propyl]piperazines—synthesis, anticonvulsant activity and relationship to inhibition of pyruvic acid oxidation □ Structure–activity relationships—*N,N'*-bis[3-(3-substituted urea)propyl]piperazines, anticonvulsant activity, rats, inhibition of pyruvic acid oxidation □ Oxidation, pyruvic acid—effect of piperazinoureas, relationship to anticonvulsant activity □ Piperazines, *N,N'*-bis[3-(3-substituted urea)propyl]—synthesis, anticonvulsant activity and relationship to inhibition of pyruvic acid oxidation

Anticonvulsant, antireserpine, and central nervous system (CNS) depressant properties exhibited by substituted piperazines (1, 2) and the inhibition of the oxidation of pyruvic acid by *N,N'*-bis[3-(3-substituted thiourea)propyl]piperazines (3) possessing anticonvulsant activity (4) led to the synthesis of some *N,N'*-bis[3-(3-substituted urea)propyl]piperazines (piperazinoureas). In the present study, attempts were made to correlate the anticonvulsant activity possessed by these piperazinoureas with their enzyme inhibitory effectiveness.

EXPERIMENTAL[1]

***N,N'*-Bis[3-(3-substituted urea)propyl]piperazines (I–XIII)**—Compounds I–XIII were prepared by refluxing a mixture of *N,N'*-bis(3-aminopropyl)piperazine[2] (0.01 mole) and the appropriate isocyanate (0.02 mole) in dry benzene on a steam bath for 2 hr. Excess benzene was removed by distillation under reduced pressure. The solid mass which separated out was collected by filtration and recrystallized from suitable solvents. These substituted piperazinoureas were characterized by their sharp melting points, elemental analyses[3], and IR spectra (Table I).

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined in mice of either sex weighing 25–30 g. The mice were divided into groups of 10, keeping the group weights as near the same as possible. Each piperazinourea was suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v). The test compound was injected in a group of 10 animals at a dose of 100 mg/kg ip.

Four hours after the administration of the piperazinourea, the mice were injected with pentylenetetrazol (90 mg/kg sc). This dose

[1] All compounds were analyzed for their carbon, hydrogen, and nitrogen content. Melting points were taken in open capillary tubes and are corrected. IR spectra were obtained with Perkin-Elmer Infracord spectrophotometer model 137 equipped with NaCl optics in KBr films in the range of 700–3500 cm^{-1}.

[2] Aldrich Chemical Co., Milwaukee, Wis.

[3] Central Drug Research Institute, Lucknow, India.

Table I—Physical Constants of *N,N'*-Bis[3-(3-substituted urea)propyl]piperazines

Compound	R	Melting Point[a]	Yield, %	Recrystallization Solvent	Formula	IR Spectra[b] (CONH), cm^{-1}	Analysis, %	
							Calc.	Found
I	C_2H_5	149°	55	Dioxane	$C_{16}H_{34}N_6O_2$	1640	C 56.14	56.00
							H 9.94	10.00
							N 24.56	24.31
II	C_6H_5	202°	90	Dioxane	$C_{24}H_{34}N_6O_2$	1640	C 65.75	66.13
							H 7.76	7.85
							N 19.17	19.43
III	*o*-CH_3—C_6H_4	227°	60	Acetic acid	$C_{26}H_{38}N_6O_2$	1650	C 66.95	66.76
							H 8.15	8.42
							N 18.02	18.42
IV	*m*-CH_3—C_6H_4	195–197°	75	Nitrobenzene	$C_{26}H_{38}N_6O_2$	1650	C 66.95	67.03
							H 8.15	8.32
							N 18.02	18.13
V	*p*-CH_3—C_6H_4	225°	85	Acetic acid	$C_{26}H_{38}N_6O_2$	1660	C 66.95	67.25
							H 8.15	7.94
							N 18.02	18.20
VI	*o*-Cl—C_6H_4	235°	60	Acetic acid	$C_{24}H_{32}Cl_2N_6O_2$	1655	C 56.80	56.66
							H 6.31	5.95
							N 16.56	16.21
VII	*m*-Cl—C_6H_4	238°	77	Acetic acid	$C_{24}H_{32}Cl_2N_6O_2$	1650	C 56.80	56.96
							H 6.31	6.33
							N 16.56	16.70
VIII	*p*-Cl—C_6H_4	203° dec.	82	Acetic acid	$C_{24}H_{32}Cl_2N_6O_2$	1655	C 56.80	56.72
							H 6.31	6.55
							N 16.56	16.36
IX	*p*-Br—C_6H_4	>270°	83	Acetic acid	$C_{24}H_{32}Br_2N_6O_2$	1660	C 48.32	48.44
							H 5.37	5.57
							N 14.09	13.96
X	*o*-OCH_3—C_6H_4	175°	62	Acetonitrile	$C_{26}H_{38}N_6O_4$	1650	C 62.65	62.40
							H 7.63	7.94
							N 16.86	16.56
XI	*p*-OCH_3—C_6H_4	196°	82	Nitrobenzene	$C_{26}H_{38}N_6O_4$	1650	C 62.65	62.70
							H 7.63	7.78
							N 16.86	16.69
XII	*o*-OC_2H_5—C_6H_4	182°	60	Dioxane	$C_{28}H_{42}N_6O_4$	1655	C 63.87	64.01
							H 7.98	8.23
							N 15.96	16.08
XIII	*p*-OC_2H_5—C_6H_4	203°	80	Nitrobenzene	$C_{28}H_{42}N_6O_4$	1640	C 63.87	63.92
							H 7.98	8.15
							N 15.96	16.09

[a] All melting points were taken in open capillary tubes and are corrected. [b] All substituted piperazinoureas showed their characteristic absorptions for CONH.

of pentylenetetrazol has been shown not only to produce convulsions in almost all untreated mice but also to exhibit 100% mortality during 24 hr (5). However, no mortality was observed during 24 hr in animals treated with 100 mg/kg of the test compounds alone.

The mice were then observed for 60 min for seizures. An episode of clonic spasm that persisted at least 5 sec was considered a threshold convulsion. Transient intermittent jerks and tremulousness were not counted. Animals devoid of threshold convulsions during the 60 min were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of these substituted piperazinoureas was represented as percent protection. In the present study, no anticonvulsant activity was observed in animals treated with 5% aqueous gum acacia solution alone. The animals were then observed for 24 hr and their mortality was recorded.

Toxicity Studies—The approximate 50% lethal dose (approximate LD_{50} values) of the substituted piperazinoureas (Table II) was determined in albino mice of either sex, weighing 20–25 g, by following the method reported earlier (6).

Determination of Respiratory Activity of Rat Brain Homogenate[4]—Male albino rats, kept on *ad libitum* diet, were used in all experiments. Rats, 100–150 g, were sacrificed by decapitation. The brains were removed immediately and homogenized in ice-cold 0.25 *M* sucrose in a Potter–Elvehjem homogenizer in a ratio of 1:9 (w/v). All incubations were carried out at 37° in the conventional Warburg manometric apparatus, using air as the gas phase (5). The oxygen uptake was measured at 10-min intervals.

Fresh rat brain homogenate (1 ml) equivalent to 100 mg wet weight was added to chilled Warburg vessels containing 6.7 m*M* magnesium sulfate, 20 m*M* sodium hydrogen phosphate buffer solution (pH 7.4), 1 m*M* adenosine monophosphate (sodium salt), 33 m*M* potassium chloride, and 500 μg of cytochrome c in a final volume of 3 ml unless otherwise stated. The central well contained 0.2 ml of 20% KOH solution. Pyruvate was used at a final concentration of 10 m*M*. It was presumed that the endogenous NAD, present in this homogenate, was sufficient for the oxidation of sodium pyruvate.

All substituted piperazinoureas were dissolved in propylene glycol (100%) and were used at a final concentration of 1×10^{-3} *M*. An equal volume of propylene glycol was added to the control vessels.

RESULTS AND DISCUSSION

In the present study, *N,N'*-bis[3-(3-substituted urea)propyl]piperazines were synthesized and evaluated for their anticonvulsant activity. The degree of protection afforded by these piperazinoureas against pentylenetetrazol-induced seizures and the 24-hr mortalities are recorded in Table II. These results indicated that the compounds possessing 70% protection (X and XIII) also provided greater protection against pentylenetetrazol-induced mortality in the experimental animals. However, such a relationship between greater anticonvulsant activity and less mortality was not

[4] Commercial chemicals were used. Sodium pyruvate, adenosine monophosphate (sodium salt), and cytochrome c were obtained from Sigma Chemical Co., St. Louis, Mo. Other common chemicals were obtained from British Drug House, Bombay, India.

Table II—Anticonvulsant Activity of *N,N'*-Bis[3-(3-substituted urea)propyl]piperazines and Their Inhibitory Effects on the Oxidation of Pyruvic Acid by Rat Brain Homogenates

Compound	Anticonvulsant Activity[a], % Protection	Pentylenetetrazol Mortality[a] after 24 hr, %	Approximate LD_{50} Values[a], mg/kg	Inhibition[b], %
I	50	50	>3200	37.5 ± 1.44
II	50	50	>3200	Nil
III	50	40	1600	20.1 ± 1.40
IV	60	30	1600	17.4 ± 1.41
V	40	20	>1600	19.5 ± 1.95
VI	40	30	>1600	15.2 ± 1.51
VII	30	40	>1600	Nil
VIII	20	20	>1600	Nil
IX	40	30	1600	Nil
X	70	10	>1600	17.5 ± 1.20
XI	40	50	>1600	31.5 ± 0.85
XII	50	40	>1600	14.2 ± 0.92
XIII	70	20	1600	52.7 ± 2.60

[a] Screening procedures for the determination of the anticonvulsant activity and the approximate LD_{50} values are described in the text. Substituted piperazinoureas were administered (100 mg/kg ip) 4 hr before the administration of pentylenetetrazol (90 mg/kg). Mortality in pentylenetetrazol-treated animals was observed for 24 hr. [b] Vessel contents and assay procedures are described in the text. Each experiment was done in duplicate, and the values are the mean values of four separate experiments with ± standard error of the mean. The oxygen uptake was measured at 10-min intervals during a 1-hr incubation. The percentage inhibition was calculated from the decrease in the oxygen uptake per 100 mg wet brain weight. The mean oxygen uptake during oxidation of pyruvic acid, in the absence of piperazinoureas, was 150.46 ± 1.41 μl/100 mg/hr. The final concentrations of pyruvic acid and substituted piperazinoureas were 10 and 1 m*M*, respectively.

sufficiently uniform (*e.g.*, Table II: 50% protection = 50 or 40% mortality; 40% protection = 20 or 30% mortality; 20% protection = 20% mortality).

Each piperazinourea was found to be devoid not only of the sedative or CNS-depressant effect but also of 24-hr mortality in the 100-mg/kg dose used in the present study. Thus, 24-hr mortality in the experimental animals accounts for the toxic effects of pentylenetetrazol. The anticonvulsant activity of these piperazinoureas ranged from 20 to 70%. Maximum activity was observed with compounds possessing *o*-CH_3—C_6H_4 (X) and *p*-OC_2H_5—C_6H_4 (XIII) as substituents at position R of their structure. Minimum protection was observed with the piperazinourea having a *p*-Cl—C_6H_4 (VIII) substituent at position R. These piperazinoureas possessed low toxicity, which was reflected by their high approximate LD_{50} values of 1600 mg/kg or higher.

The inhibitory effects of substituted piperazinoureas on the oxidation of pyruvic acid by rat brain homogenates are presented in Table II. The degree of inhibition by these compounds, when used at a final concentration of 1×10^{-3} *M*, ranged from 14.2 to 52.7%; piperazinoureas possessing C_6H_5, *m*-Cl—C_6H_4, *p*-Cl—C_6H_4, and *m*-Br—C_6H_4 substituents at position R of their structure (II, VII, VIII, and IX, respectively) were devoid of the inhibitory property. Maximum inhibition was observed with the compound possessing the *p*-OC_2H_5—C_6H_4 substituent (XIII), while the piperazinourea possessing *o*-OC_2H_5—C_6H_4 substituent (XII) at position R was the least effective inhibitor.

Inhibition of certain metabolic processes in the brain has been proposed as a biochemical basis for the mechanism of action of various CNS depressants (7, 8). A parallelism was observed between greater *in vivo* hypnotic activity of some compounds and their ability to cause greater *in vitro* inhibition of respiration (9, 10). In spite of the maximum inhibition observed with Compound XIII, which also possessed maximum anticonvulsant activity, the present study failed to provide any definite correlation between the anticonvulsant activity possessed by these substituted piperazinoureas and their ability to inhibit the oxidation of pyruvic acid by rat brain homogenates.

Similar results were reported earlier with 3,4,5-trimethoxybenzamides (11). It is hoped that further detailed pharmacological and toxicological studies and investigations on the activity of substituted piperazinoureas on other enzyme systems will reflect a biochemical basis for their anticonvulsant activity.

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ACKNOWLEDGMENTS AND ADDRESSES

Received March 28, 1974, from the **Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow 3, India,* and the ‡*Department of Chemistry, University of North Dakota, Grand Forks, ND 58201*

Accepted for publication September 11, 1974.

Supported in part by the Council of Scientific and Industrial Research, New Delhi, India, U.S. Public Health Service National Institutes of Health Grant 1 T01 HL 05939-01A1, and U.S. Public Health Service Research Career Development Award 5-K04 GM09888 to V. I. Stenberg.

The authors thank Professor K. P. Bhargava and Professor Roland G. Severson for their advice and encouragement. Grateful acknowledgment is made to Dr. M. L. Dhar and Dr. Nitya Anand of the Central Drug Research Institute, Lucknow, India, for providing facilities for microanalyses and IR spectroscopy, and to the Council of Scientific and Industrial Research, New Delhi, India, for providing a Senior Research Fellowship to A. K. Chaturvedi.

ˣ To whom inquiries should be directed. Present address: Department of Physiology and Pharmacology, School of Medicine, University of North Dakota, Grand Forks, ND 58201