Anticonvulsant Activity and Selective Inhibition of NAD-Dependent Oxidations in Rat Brain Homogenates by Newer Mercaptotriazoles

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Abstract □ Eight 1-(2,6-dimethylphenoxyacetyl)-4-(substituted phenyl)thiosemicarbazides were cyclized to the corresponding 5-(2,6-dimethylphenoxymethyl)-4-(substituted phenyl)-3-mercapto-1,2,-4(4*H*)-triazoles and 5-(2,6-dimethylphenoxymethyl)-4-(substituted phenyl)-3-[1,2,4(4*H*)-triazolethioglycolic] acids. These compounds were characterized by their sharp melting points, elemental analyses, and IR spectra and were evaluated for anticonvulsant activity. The degree of protection (range) provided by these thiosemicarbazides, triazoles, and triazolethioglycolic acids at a dose of 100 mg/kg ip against pentylenetetrazol (90 mg/kg sc)-induced convulsions in mice was 10–50, 20–80, and 10–70%, respectively, where cyclization to triazoles increased anticonvulsant activity of the precursor thiosemicarbazides. Increased protection by these compounds against convulsions was generally associated with decreased 24-hr pentylenetetrazol-induced mortality. These compounds exhibited selective *in vitro* inhibition of nicotinamide adenine dinucleotide (NAD)-dependent oxidation of pyruvate, α-ketoglutarate, and NADH by rat brain homogenates while NAD-independent oxidation of succinate remained unaltered. The presence of added NAD to the reaction mixture during *in vitro* oxidation of pyruvic acid not only increased the respiratory activity of rat brain homogenates but also decreased the inhibitory effectiveness of thiosemicarbazides, triazoles, and triazolethioglycolic acids. The degree of selective inhibition of NAD-dependent oxidations was unrelated to their anticonvulsant activity.

Keyphrases □ Thiosemicarbazides, various—synthesized, evaluated for anticonvulsant activity and effect on NAD-dependent oxidations, rats □ Mercaptotriazoles, various—synthesized, evaluated for anticonvulsant activity and effect on NAD-dependent oxidations, rats □ Triazolethioglycolic acids, various—synthesized, evaluated for anticonvulsant activity and effect on NAD-dependent oxidations, rats □ Anticonvulsant activity—evaluated in various thiosemicarbazides, mercaptotriazoles, and triazolethioglycolic acids, rats □ NAD-dependent oxidations—effect of various thiosemicarbazides, mercaptotriazoles, and triazolethioglycolic acids, rat brain homogenates □ Oxidations, NAD dependent—effect of various thiosemicarbazides, mercaptotriazoles, and triazolethioglycolic acids, rat brain homogenates □ Structure–activity relationships—various thiosemicarbazides, mercaptotriazoles, and triazolethioglycolic acids evaluated for anticonvulsant activity and effect on NAD-dependent oxidations, rats

Interest has been focused on the pharmacology of 1-phenyl-1,2,4-triazoles and 4-phenyl-1,2,4-triazoles (1). Earlier studies indicated central nervous system depressant (2), analgesic (3), and anti-inflammatory (4, 5) properties of 1,2,4(4*H*)-triazoles. Furthermore, 1,4-disubstituted thiosemicarbazides, the precursors for the synthesis of cyclized triazoles, possess a broad spectrum of biological activities (6, 7). Anticonvulsant compounds also cause selective inhibition of nicotinamide adenine dinucleotide (NAD)-dependent oxidations (8).

These observations prompted synthesis of eight 1-(2,6-dimethylphenoxyacetyl)-4-(substituted phenyl)-thiosemicarbazides and their corresponding cyclized 5-(2,6-dimethylphenoxymethyl)-4-(substituted phenyl)-3-mercapto-1,2,4(4*H*)-triazoles. The substituted triazoles were further converted into 5-(2,6-dimethylphenoxymethyl)-4-(substituted phenyl)-3-[1,2,4(4*H*)-triazolethioglycolic] acids. The effects of all compounds were determined on the respiratory activity of rat brain homogenates in an attempt to elucidate the biochemical basis for their anticonvulsant activity.

CHEMISTRY

The various substituted 1,2,4(4*H*)-triazoles were synthesized by following the reactions outlined in Scheme I. Conversion of 2,6-dimethylphenol (I) to ethyl 2,6-dimethylphenoxyacetate (II) was carried out to synthesize 2,6-dimethylphenoxyacetohydrazide (III) through refluxing in ethanol with 99–100% hydrazine hydrate. Substituted thiosemicarbazides (IV–XI, Table I), prepared by the reaction of III with the appropriate aryl isothiocyanate, were cyclized to the corresponding mercaptotriazoles (XII–XIX, Table II) in 2 *N* NaOH. Condensation of the substituted triazoles with monochloroacetic acid resulted in the formation of substituted 3-triazolethioglycolic acids (XX–XXVII, Table III). All compounds were characterized by their sharp melting points, elemental analyses, and IR spectra.

EXPERIMENTAL[1]

Ethyl 2,6-Dimethylphenoxyacetate (II)—Equimolar quantities of I (0.2 mole), ethyl chloroacetate (0.2 mole), and potassium carbonate (0.25 mole) in dry acetone (60 ml) were refluxed under anhydrous conditions for 12 hr. The reaction mixture was filtered, and the filtrate was poured over 100 ml of ice-cold water. The separated ester was extracted with ether and dried over anhydrous magnesium sulfate. Excess ether was removed by distillation, the remaining liquid fraction was subjected to fractional distillation, and the fraction boiling at 270° was collected, yield 33.3 g (80%).

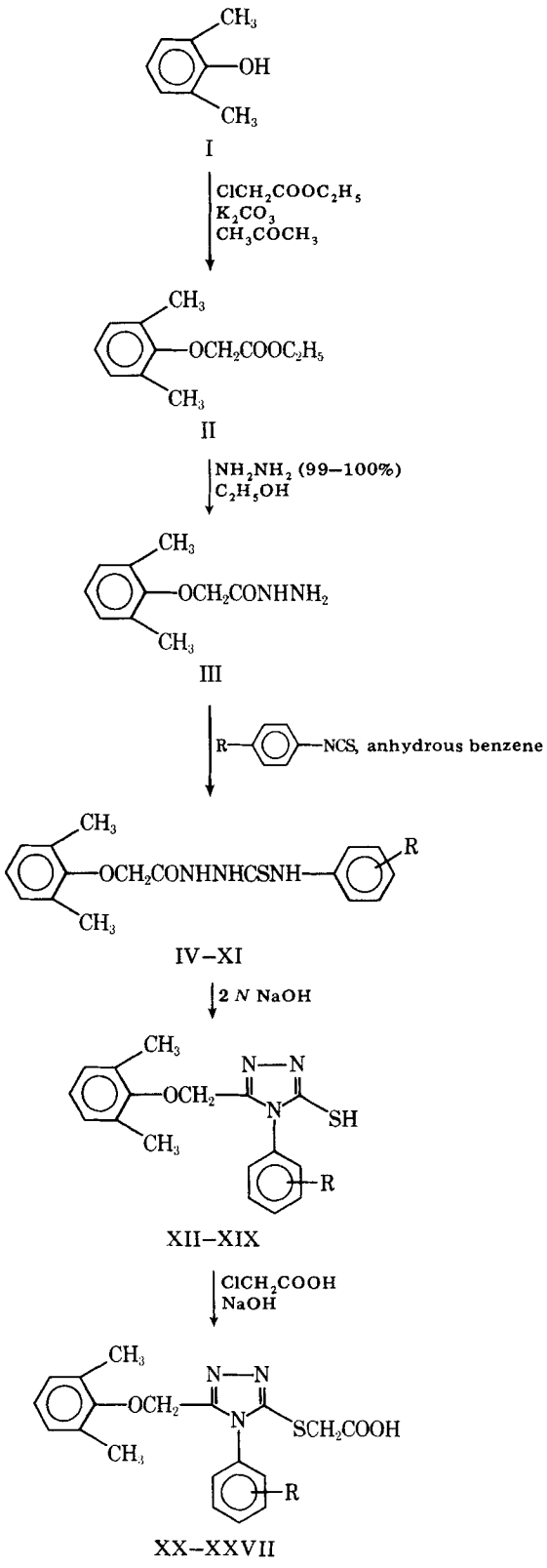
2,6-Dimethylphenoxyacetohydrazide (III)—To a solution of 0.15 mole (31.2 g) of II in absolute ethanol (50 ml) was added 10 g of 99–100% hydrazine hydrate, and the mixture was refluxed under anhydrous conditions on a water bath for 4 hr. A white solid mass, which separated on cooling, was collected by filtration and recrystallized from ethanol into white crystalline solid, mp 92°, yield 17.4 g (60%).

Anal.—Calc. for $C_{10}H_{14}N_2O_2$: C, 61.85; H, 7.21; N, 14.63. Found: C, 61.40; H, 7.36; N, 14.98.

1-(2,6-Dimethylphenoxyacetyl)-4-(substituted Phenyl)-thiosemicarbazides (IV–XI)—Equimolar quantities of III (0.02 mole) and the appropriate aryl isothiocyanate (0.02 mole) were mixed in dry benzene, and the mixture was refluxed on a steam bath for 2 hr. Excess benzene was removed by distillation. The separated solid was collected by filtration, washed with a cold ethanol–water mixture, dried, and recrystallized from ethanol. The various substituted thiosemicarbazides (Table I) were characterized by their sharp melting points and elemental analyses.

5-(2,6-Dimethylphenoxymethyl)-4-(substituted Phenyl)-3-mercapto-1,2,4(4*H*)-triazole (XII–XIX)—A solution of 1-(2,6-dimethylphenoxyacetyl)-4-(substituted phenyl)thiosemicarbazide (0.01 mole) in 2 *N* NaOH was refluxed over a free flame for 2–3 hr. After cooling, the mixture was filtered; the filtrate was acidified with dilute hydrochloric acid until complete precipitation occurred (9, 10). The separated solid was collected by filtration, washed with water, dried, and recrystallized from ethanol. Various substituted mercaptotriazoles were characterized by their sharp melting points and elemental analyses (Table II).

[1] All compounds were analyzed for their carbon, hydrogen, and nitrogen content. Melting points were taken in open capillary tubes with a partial immersion thermometer and are corrected. IR spectra provided support for the structures of these compounds.



Scheme I

5-(2,6-Dimethylphenoxymethyl)-4-(substituted Phenyl)-3-[1,2,4(4*H*)-triazolethioglycolic] Acids (XX–XXVII)—A mixture of the appropriate substituted mercaptotriazole (0.005 mole), chloroacetic acid (0.005 mole), and sodium hydroxide (0.01 mole) was refluxed for 3 hr. The mixture was cooled and acidified with dilute hydrochloric acid. The solid mass, which separated slowly from the acid solution, was collected by filtration and recrystallized from ethanol (11) (Table III).

Determination of Respiratory Activity of Rat Brain Homogenates[2]—Male albino rats, 100–150 g, were kept on an *ad libitum* diet until they were sacrificed by decapitation. The brains were removed immediately and homogenized[3] in ice-cold 0.25 *M* sucrose 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 (12). The oxygen uptake was measured at 10-min intervals.

Fresh brain homogenate (1 ml) equivalent to 100 mg wet brain weight was added to the chilled Warburg vessels containing 6.7 m*M* magnesium sulfate, 20 m*M* dibasic sodium 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, α-ketoglutarate, and succinate were used at a final concentration of 10 m*M*; NAD and NADH were used at 0.5 m*M*. It was presumed that the endogenous NAD, present in brain homogenates, was sufficient for the cellular respiratory activity of rat brain homogenates.

All test compounds were dissolved in propylene glycol (100%) and were used at a final concentration of 2 m*M*. An equal volume of propylene glycol was added to the control vessels.

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined (13) in mice of both sexes weighing 25–30 g. The mice were divided into groups of 10, keeping the group weights as near the same as possible. The test compounds were suspended in 5% aqueous gum acacia (1% w/v) and injected in a group of 10 mice each at a dose of 100 mg/kg ip. Four hours after the administration of the test compound, the mice were injected with 90 mg/kg sc of pentylenetetrazol. This dose of pentylenetetrazol not only produced convulsions in almost all untreated mice but also caused 100% mortality during the 24-hr period. No mortality was found in mice treated with 100 mg/kg alone of the test compounds, and the animals were devoid of any behavioral effects.

The occurrence of seizures was observed for 60 min. An episode of clonic spasm that persisted for a minimum of 5 sec after administration of pentylenetetrazol was considered a threshold convulsion. Transient intermittent jerks and tremulousness were not counted. Animals devoid of threshold convulsions during 60 min were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of the test compounds was represented as the percent protection. The mice were then observed for 24 hr, and their mortality was recorded.

RESULTS AND DISCUSSION

In the present study, the anticonvulsant activity possessed by thiosemicarbazides, triazoles, and triazolethioglycolic acids was reflected by their ability to provide 10–50% (Table I), 20–80% (Table II), and 10–70% (Table III) protection, respectively, against pentylenetetrazol (90 mg/kg sc)-induced convulsions in mice at a dose of 100 mg/kg ip. Compounds V, X, and XXII were devoid of anticonvulsant activity, since no protection was observed at 100 mg/kg. These results indicated an increase in anticonvulsant activity by cyclization of the precursor thiosemicarbazides into the corresponding triazoles. Further conversion of the triazoles into the corresponding triazolethioglycolic acids in most cases caused a decrease in their anticonvulsant activity; however, XXIV and XXV provided greater protection than their precursors (XVI and XVII).

These results did not provide any specific requirements in the molecular structure of these compounds to warrant structure–activity relationships with respect to the anticonvulsant activity. In addition, the anticonvulsant activity of some substituted mercaptotriazoles alone was comparable to that of methaqualone and meprobamate, which provided 60 and 80% protection, respectively, at 100 mg/kg ip against pentylenetetrazol-induced convulsions in mice under similar experimental conditions (14).

All thiosemicarbazides, triazoles, and triazolethioglycolic acids selectivity inhibited NAD-dependent oxidation of pyruvate, α-ketoglutarate, and NADH by rat brain homogenates at 2 m*M* (Table IV). The degree of inhibition of pyruvic acid oxidation ranged from 35.4 to 93.7, from 18.5 to 62.3, and from 30.5 to 90.8% with thiosemicarbazides, triazoles, and triazolethioglycolic acids, respectively. Similar inhibition of the oxidation of α-ketoglutarate was 30.5–54.2, 14.4–46, and 37.2–64.7%, respectively. Of NADH, it was 36.2–60.2, 14.1–89.2, and 45.6–67.5% by thiosemicarbazides, triazoles, and triazolethioglycolic acids, respectively. Oxidation of succinate remained unaltered in the presence of these compounds.

[2] Commercial chemicals were used. Sodium pyruvate, sodium α-ketoglutarate, NADH, sodium succinate, adenosine monophosphate (sodium salt), and cytochrome c were obtained from Sigma Chemical Co., St. Louis, Mo.

[3] Potter–Elvehjem.

Table I—1-(2,6-Dimethylphenoxyacetyl)-4-(substituted Phenyl)thiosemicarbazides and Their Anticonvulsant Activity

Compound	R	Melting Point	Yield, %	Molecular Formula	Analysis, %	Calc.	Found	Anticonvulsant Activity[a], % Protection	Pentylenetetrazol Mortality[b], %
IV	H	166°	73	$C_{17}H_{19}N_3O_2S$	C	62.00	62.20	30	50
					H	5.77	5.78		
					N	12.76	12.60		
V	2-CH_3	150°	70	$C_{18}H_{21}N_3O_2S$	C	62.97	62.80	Nil	90
					H	6.12	6.32		
					N	12.24	12.13		
VI	4-CH_3[c]	168°	68	$C_{18}H_{21}N_3O_2S$	C	62.97	62.86	50	70
					H	6.12	6.32		
					N	12.24	12.25		
VII	2,4-$(CH_3)_2$	108°	81	$C_{19}H_{23}N_3O_2S$	C	63.86	63.72	30	50
					H	6.44	6.54		
					N	11.76	11.80		
VIII	2,6-$(CH_3)_2$	170°	60	$C_{19}H_{23}N_3O_2S$	C	63.86	63.72	10	100
					H	6.44	6.46		
					N	11.76	11.60		
IX	2-OCH_3	144°	68	$C_{18}H_{21}N_3O_3S$	C	60.16	60.20	50	50
					H	5.84	5.60		
					N	11.69	11.70		
X	4-OCH_3	142°	66	$C_{18}H_{21}N_3O_3S$	C	60.16	60.26	Nil	90
					H	5.84	5.76		
					N	11.69	11.72		
XI	4-Cl	174°	65	$C_{17}H_{18}ClN_3O_2S$	C	56.12	56.29	30	10
					H	4.95	5.02		
					N	11.55	11.64		

[a] Anticonvulsant activity was determined at 100 mg/kg ip as described under *Experimental*. [b] Represents mortality over 24 hr in each group of animals administered pentylenetetrazol (90 mg/kg sc). [c] The presence of characteristic bands of C=O (attached to nitrogen), C=S (flanked by nitrogen), and NH groups at 1695, 1538, and 3344 cm^{-1}, respectively, in the IR spectrum of VI provided further support for the structure of substituted thiosemicarbazides.

Addition of NAD during the oxidation of pyruvate not only increased the respiratory activity of rat brain homogenates but also decreased the ability of these compounds to inhibit pyruvate oxidation, as reported earlier with quinazolones (15), β-aminoketones (12), and thiazolidones (16). The degree of inhibition of 35.4–93.7, 18.5–62.3, and 30.5–90.8% observed during the oxidation of pyruvate by thiosemicarbazides, triazoles, and triazolethioglycolic acids was reduced to 26.2–82.3, 10.7–52.7, and 25.5–79.3%, respectively, in the presence of added NAD. These results provided evidence for a possible competition between thiosemicarbazides, triazoles, and triazolethioglycolic acids and NAD for the active site(s) on the enzyme molecule. Cyclization to triazoles caused a significant decrease in the ability of triazoles to inhibit NAD-dependent oxidations as compared to precursor thiosemicarbazides. Further conversion of triazoles into triazolethioglycolic acids increased the inhibitory effectiveness of the latter compounds to inhibit respiratory activity of rat brain homogenates with pyruvate, α-ketoglutarate, and NADH as the substrates.

As was observed with the anticonvulsant activity possessed by these compounds, their selective inhibition of NAD-dependent oxidations was unrelated to their structure; a definite structure–activity relationship was not exhibited (Table IV). The ability of these thiosemicarbazides, triazoles, and triazolethioglycolic acids to inhibit oxidation of pyruvate, α-ketoglutarate, and NADH provides evidence regarding the possible inactivation of the electron-transfer process in the electron-transport chain by their actions presumably at the site of transfer of electrons from NADH to flavine adenine dinucleotide.

Contrary to the increase in the anticonvulsant activity observed after cyclization of thiosemicarbazides into triazoles, the results indicated a decrease in the ability of triazoles to inhibit NAD-dependent oxidations by rat brain homogenates. Thus, selective inhibition of the respiratory

Table II—5-(2,6-Dimethylphenoxymethyl)-4-(substituted Phenyl)-3-mercapto-1,2,4(4*H*)-triazoles and Their Anticonvulsant Activity

Compound	R	Melting Point	Yield, %	Molecular Formula	Analysis, %	Calc.	Found	Anticonvulsant Activity[a], % Protection	Pentylenetetrazol Mortality[a], %
XII	H	246°	77	$C_{17}H_{17}N_3OS$	C	65.59	65.40	80	20
					H	5.46	5.23		
					N	13.50	13.26		
XIII	2-CH_3	201°	80	$C_{18}H_{19}N_3OS$	C	66.46	66.72	60	40
					H	5.84	5.76		
					N	12.92	12.90		
XIV	4-CH_3[b]	182°	58	$C_{18}H_{19}N_3OS$	C	66.46	66.42	50	50
					H	5.84	5.62		
					N	12.92	12.96		
XV	2,4-$(CH_3)_2$	204°	70	$C_{19}H_{21}N_3OS$	C	67.25	66.96	70	20
					H	6.19	6.32		
					N	12.39	12.50		
XVI	2,6-$(CH_3)_2$	220°	56	$C_{19}H_{21}N_3OS$	C	67.25	67.50	50	60
					H	6.19	6.38		
					N	12.39	12.40		
XVII	2-OCH_3	198°	64	$C_{18}H_{19}N_3O_2S$	C	63.34	63.50	40	70
					H	5.57	5.60		
					N	12.31	12.56		
XVIII	4-OCH_3	184°	67	$C_{18}H_{19}N_3O_2S$	C	63.34	63.24	50	70
					H	5.57	5.60		
					N	12.31	12.52		
XIX	4-Cl	194°	68	$C_{17}H_{16}ClN_3OS$	C	59.04	58.96	20	60
					H	4.63	4.72		
					N	12.15	12.25		

[a] Screening procedures were as indicated in Table I. [b] The presence of the characteristic band of C=N (1610 cm^{-1}) and the absence of the C=O stretching band in the IR spectrum of XIV provided further support for the structure of substituted mercaptotriazoles.

Table III—5-(2,6-Dimethylphenoxymethyl)-4-(substituted Phenyl)-1,2,4(4*H*)triazole-3-yl-thioglycolic Acids and Their Anticonvulsant Activity

Compound	R	Melting Point	Yield, %	Molecular Formula	Analysis, %	Calc.	Found	Anticonvulsant Activity[a], % Protection	Pentylenetetrazol Mortality[a], %
XX	H	152°	60	$C_{19}H_{19}N_3O_3S$	C	61.79	61.60	10	70
					H	5.14	5.20		
					N	11.38	11.42		
XXI	2-CH_3[b]	200°	65	$C_{20}H_{21}N_3O_3S$	C	62.66	62.40	30	70
					H	5.48	5.60		
					N	10.96	11.20		
XXII	4-CH_3	172°	70	$C_{20}N_{21}N_3O_3S$	C	62.66	62.44	Nil	50
					H	5.48	5.30		
					N	10.96	10.68		
XXIII	2,4-$(CH_3)_2$	144°	65	$C_{21}H_{23}N_3O_3S$	C	63.47	63.27	10	70
					H	5.79	5.82		
					N	10.57	10.60		
XXIV	2,6-$(CH_3)_2$	218°	60	$C_{21}H_{23}N_3O_4S$	C	63.47	63.30	70	Nil
					H	5.79	5.82		
					N	10.57	10.62		
XXV	2-OCH_3	168°	62	$C_{20}H_{21}N_3O_4S$	C	60.15	60.25	50	10
					H	5.26	5.40		
					N	10.52	10.72		
XXVI	4-OCH_3	150°	55	$C_{20}H_{21}N_3O_4S$	C	60.15	60.28	10	50
					H	5.26	5.30		
					N	10.52	10.40		
XXVII	4-Cl	138°	68	$C_{19}H_{18}ClN_3O_3S$	C	56.50	56.40	10	50
					H	4.46	4.52		
					N	10.40	10.60		

[a] Screening procedures were as indicated in Table I. [b] The presence of characteristic bands of C=O and C=N groups at 1739 and 1600 cm^{-1}, respectively, in the IR spectrum of XXI provided further support for the structure of substituted triazolethioglycolic acids.

Table IV—Selective Inhibition (Percent) of Respiratory Activity of Rat Brain Homogenates by Thiosemicarbazides, Triazoles, and Triazolethioglycolic Acids[a]

Compound	Pyruvate Oxidation: Absence of NAD	Pyruvate Oxidation: Presence of NAD	α-Ketoglutarate Oxidation	NADH Oxidation
		Thiosemicarbazides		
IV	55.2 ± 1.3	43.8 ± 0.9	54.2 ± 1.2	54.9 ± 1.5
V	82.7 ± 0.9	72.9 ± 1.3	43.1 ± 1.4	44.1 ± 1.7
VI	93.7 ± 0.9	82.3 ± 1.4	41.7 ± 0.9	36.2 ± 0.9
VII	83.9 ± 1.3	69.5 ± 1.1	33.4 ± 1.5	39.4 ± 1.0
VIII	73.3 ± 1.3	61.3 ± 1.8	44.9 ± 1.1	46.1 ± 1.2
IX	64.1 ± 0.8	58.3 ± 1.4	51.3 ± 1.3	60.2 ± 1.1
X	35.4 ± 1.0	26.2 ± 0.9	30.5 ± 1.4	38.6 ± 0.7
XI	64.3 ± 0.8	52.4 ± 1.3	44.3 ± 1.1	50.5 ± 1.5
		Triazoles		
XII	51.1 ± 1.5	34.3 ± 0.8	20.5 ± 0.3	16.5 ± 0.5
XIII	18.5 ± 0.8	10.7 ± 1.4	14.4 ± 0.9	29.1 ± 1.1
XIV	63.6 ± 1.3	52.7 ± 0.8	37.3 ± 1.2	89.2 ± 1.5
XV	40.2 ± 0.9	29.1 ± 1.3	46.0 ± 1.0	57.5 ± 1.2
XVI	26.2 ± 0.6	18.4 ± 1.3	23.0 ± 1.3	14.1 ± 0.5
XVII	62.3 ± 1.7	50.5 ± 1.5	41.5 ± 1.3	59.5 ± 1.4
XVIII	29.1 ± 0.6	17.6 ± 1.2	16.3 ± 1.0	26.5 ± 1.3
XIX	48.1 ± 1.2	30.5 ± 0.9	30.4 ± 1.3	67.4 ± 1.6
		Triazolethioglycolic Acids		
XX	64.0 ± 1.3	50.9 ± 1.2	55.1 ± 1.5	66.2 ± 1.4
XXI	75.0 ± 1.3	64.4 ± 1.3	64.7 ± 1.2	50.3 ± 1.3
XXII	90.0 ± 0.8	70.5 ± 1.4	59.5 ± 1.4	59.8 ± 1.5
XXIII	87.5 ± 1.4	72.4 ± 1.3	59.5 ± 1.2	67.5 ± 1.2
XXIV	90.8 ± 2.2	79.3 ± 1.9	46.4 ± 1.3	57.2 ± 1.3
XXV	56.1 ± 1.4	40.8 ± 1.5	62.1 ± 1.4	49.2 ± 1.5
XXVI	30.5 ± 0.3	25.5 ± 1.0	58.2 ± 1.8	59.9 ± 1.3
XXVII	67.7 ± 1.4	46.5 ± 0.4	37.2 ± 1.3	45.6 ± 1.4

[a] Vessel contents and assay procedures were as described in the test. All values are the mean of three duplicate experiments. The percent inhibition and standard errors (*SE*) were calculated from the decrease in the oxygen uptake per 100 mg of wet brain weight per hour. The final concentration of pyruvate, α-ketoglutarate, and succinate was 10 m*M*; the final concentration of NAD and NADH was 0.5 m*M*. All thiosemicarbazides, triazoles, and triazolethioglycolic acids were used at 2 m*M*. The values of oxygen uptake in the control experiments during oxidation of pyruvate, pyruvate plus NAD, α-ketoglutarate, NADH, and succinate were 71.6 ± 1.3, 83 ± 2.2, 96 ± 1.8, 63.3 ± 1.1, and 163 ± 2.8 μl, respectively. The inhibition of the oxidation of succinate by these compounds was nil.

activity cannot account for the biochemical basis of the anticonvulsant activity of thiosemicarbazides, triazoles, and triazolethioglycolic acids.

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Interaction of Hydrocortisone with Model Membranes Containing Phospholipid and Cholesterol

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Abstract □ Pure and mixed monolayers of lecithin and cholesterol were spread on substrates of dissolved hydrocortisone at 25 and 37°. The presence of hydrocortisone increased the surface pressure of dipalmitoyl and egg lecithin films that were in head contact. The increase in surface pressure was dependent on steroid concentration. There were no significant interactions with coherent cholesterol monolayers. Penetration of hydrocortisone was decreased by the addition of cholesterol to the monolayer system. These model membrane systems indicate that hydrocortisone interacts with the hydrated polar head group of the phospholipid and not with films whose molecules are in hydrocarbon tail contact.

Keyphrases □ Hydrocortisone—interaction with pure and mixed monolayers of lecithin and cholesterol □ Monolayers—lecithin and cholesterol, pure and mixed, interaction with hydrocortisone □ Lecithin—monolayers, pure and mixed with cholesterol, interaction with hydrocortisone □ Cholesterol—monolayers, pure and mixed with lecithin, interaction with hydrocortisone □ Membranes, model—pure and mixed monolayers of lecithin and cholesterol, interaction with hydrocortisone

Several literature reports have drawn attention to the possibility that the biological activity of certain steroids is due to an interaction with biological membranes (1–5). In a review of steroids and cell surfaces, Willmer (1) proposed a mechanism of steroid activity based on the penetration of steroid molecules between the hydrocarbon tails of membrane phospholipids. Erythrocytes were more resistant to lysis in hypotonic solution when steroids were present in low concentration (2). In high concentration, the steroids themselves caused lysis or precipitation. Another study (3) showed that the corticosteroids tended to stabilize lysosomes at pharmacological concentration (10^{-4}–10^{-6} *M*) but to lyse them at higher concentrations. Cortisone and hydrocortisone exerted a protective effect on the membranes of erythrocytes (4) and rat liver cells (5).

BACKGROUND

One approach to an understanding of steroid–membrane interactions is through model membrane systems. Monomolecular films provide an organized interfacial structure believed to be similar to that found in biological membranes. Studies of drug penetration into monolayers containing components of natural membranes have been useful in explaining the mode of action of many drugs (6, 7).

Current concepts of cellular membranes suggest the existence of a fluid mosaic structure of globular proteins embedded in, and partially protruding from, an organized but discontinuous lipid layer (8). The site of attachment for membrane-active steroids might be protein or lipid. But membrane proteins have not been well characterized. Because of the availability of pure lipid substances known to be components of biological membranes and in view of Willmer's hypothesis (1), it was decided to use monolayers containing lecithins and cholesterol as membrane models.

Few studies on the effect of steroids on monolayers have been reported. Progesterone penetrated monolayers of cholesterol and dipalmitoyl lecithin to some extent (9). Cortisone had little effect on monolayers of stearic acid or cholesterol (10), and the properties of stearyl alcohol monolayers were not affected by the presence of dissolved steroids (11).

Progesterone, testosterone, etiocholanolone, and androsterone had little effect on the surface pressure of lipid monolayers in the condensed state (12), but the surface potential of every monolayer was lowered by the same amount in the presence of a given steroid regardless of the nature of the polar group. This finding was attributed to alteration of the structure of water beneath the monolayers by the steroids. Similar changes in surface potential were caused by hydrocortisone in the presence of lecithin and cholesterol monolayers (13). However, the observed changes were an artifact of the experimental procedure and not a result of changes in water structure.

This report describes the effect of hydrocortisone (I) on model membranes composed of monolayers containing dipalmitoyl (II) and egg (III) lecithins and cholesterol (IV). The molecular weights (in daltons) used