Anticonvulsant Activity and Selective Inhibition of Nicotinamide Adenine Dinucleotide-Dependent Oxidations by 1,3,5-Trisubstituted Pyrazolines

S. P. SINGH *, A. CHAUDHARI *, JAYANTI P. BARTHWAL *, and SURENDRA S. PARMAR *‡x

Abstract □ Several 3-(3-acetoamino)phenyl-1,5-substituted phenyl-Δ^2-pyrazolines were synthesized and evaluated for their anticonvulsant activity. All substituted pyrazolines exhibited anticonvulsant activity, which was reflected by 30–80% protection observed against pentylenetetrazol-induced seizures. Most of these substituted pyrazolines inhibited selectively the *in vitro* oxidation of substrates requiring nicotinamide adenine dinucleotide (NAD dependent) by rat brain homogenates. In the present study, the anticonvulsant activity possessed by these substituted pyrazolines was found to bear no relationship with their ability to inhibit selectively the NAD-dependent oxidations.

Keyphrases □ Pyrazolines, 1,3,5-trisubstituted—synthesis, anticonvulsant activity, relationship to NAD-dependent oxidations □ Structure–activity relationships—pyrazolines–anticonvulsant activity, rats □ Anticonvulsant activity—1,3,5-trisubstituted pyrazolines □ Nicotinamide adenine dinucleotide-dependent oxidations—inhibition by 1,3,5-trisubstituted pyrazolines

Diverse pharmacological properties exhibited by pyrazole derivatives include tranquilizing, psychoanaleptic, muscle relaxant (1), hypnotic (2), and anticonvulsant (3, 4) activities. Furthermore, selective inhibition of nicotinamide adenine dinucleotide- (NAD) dependent oxidation of pyruvic acid and substrates of the tricarboxylic acid cycle by 2-methyl-3-*o*-tolyl-4-quinazolone (5, 6) possessing hypnotic (7) and anticonvulsant (8) properties led to the synthesis of some 1,3,5-trisubstituted pyrazolines.

In the present study, substituted pyrazolines were synthesized as possible anticonvulsants to investigate correlation between anticonvulsant activity possessed by these pyrazolines with their ability to inhibit the respiratory activity of rat brain homogenates as a basis of their biochemical mechanism of action.

EXPERIMENTAL[1]

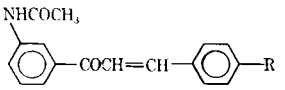
3-Acetoaminoacetophenone—This compound was prepared by heating 3-aminoacetophenone with acetic anhydride according to the method reported earlier (9).

Substituted Benzal-3-acetoaminoacetophenones—3-Acetoaminoacetophenone (0.022 mole) was gradually added, with continuous stirring, to a solution of sodium hydroxide (0.027 mole) in 12 ml of water and 20 ml of ethanol. To this mixture was added the appropriate aldehyde (0.022 mole) with stirring, and the temperature was maintained at 25°. The mixture was stirred vigorously until it resulted into a thick mass and the stirring was no longer effective, and it was kept overnight in a refrigerator.

The solid product which separated was filtered, washed with water until the washings were neutral to litmus and then with 20 ml of ice-cold ethanol, dried, and recrystallized from ethanol.

[1] *All compounds were analyzed for their carbon, hydrogen, and nitrogen content. Melting points were taken in open capillary tubes with partial immersion thermometer and are corrected.*

Table I—Physical Constants of Substituted Benzal-3-acetoaminoacetophenones



R	Melting Point[a]	Yield, %	Formula	Analysis, %	Calc.	Found
H	166°	80	$C_{17}H_{15}NO_2$	C	76.98	76.59
				H	5.66	5.33
				N	5.28	5.21
Cl	180°	77	$C_{17}H_{14}ClNO_2$	C	68.11	68.25
				H	4.67	4.68
				N	4.67	4.63
					4.67	
OCH_3	154°	85	$C_{18}H_{17}NO_3$	C	73.22	73.37
				H	5.76	5.82
				N	4.74	4.80

[a] All melting points were taken in open capillary tubes and are corrected.

These substituted benzal-3-acetoaminoacetophenones were characterized by their sharp melting points and elemental analyses (Table I).

3-(3-Acetoamino)phenyl-1,5-substituted Phenyl-Δ^2-pyrazolines—To a solution of a suitable phenylhydrazine (0.003 mole) in 10 ml of acetic acid was added the appropriate substituted benzal-3-acetoaminoacetophenone (0.003 mole), and the mixture was refluxed on a free flame for 3 hr. The resultant solution was cooled and diluted with water. The solid which separated was filtered, washed with water, dried, and recrystallized from ethanol. All pyrazolines (Table II) were characterized by their sharp melting points and elemental analyses.

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined against pentylenetetrazol-induced convulsions in mice of either sex weighing between 25 and 30 g. The mice were divided into groups of 10, keeping the group weights as near the same as possible. All compounds were suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v). The test compounds were administered in a dose of 100 mg/kg ip to a group of 10 animals.

Four hours after the administration of the test compounds, the mice were injected with pentylenetetrazol (90 mg/kg sc). This dose of pentylenetetrazol was shown to produce convulsions in almost all untreated mice, and the mice were also found to exhibit 100% mortality during 24 hr. No mortality was observed during 24 hr in animals treated with 100 mg/kg of the substituted pyrazolines alone.

The mice were observed 60 min for the occurrence of seizures. An episode of clonic spasm persisting for 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 period were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of the substituted pyrazolines was represented as percent protection. The animals were then observed for 24 hr and their mortality was recorded.

Assay of Respiratory Activity of Rat Brain Homogenate[2]—

[2] Commercial chemicals were used in the present study. Sodium pyruvate, monosodium α-ketoglutarate, sodium β-hydroxybutyrate, NADH, sodium succinate, adenosine monophosphate (sodium salt), and cytochrome c were obtained from Sigma Chemical Co., St. Louis, Mo. Other common chemicals were obtained from the British Drug House, Bombay, India.

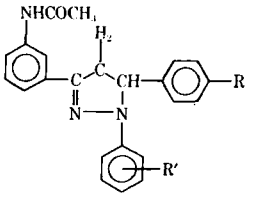


Table II—Physical Constants of 3-(3-Acetoamino)phenyl-1,5-substituted Phenyl-Δ^2-pyrazolines

Compound	R	R′	Melting Point[a]	Yield, %	Formula	Analysis, %	Calc.	Found
I	H	H	125°	72	$C_{23}H_{21}N_3O$	C	77.46	77.52
						H	5.91	5.88
						N	11.83	11.79
II	H	2-NO_2	100°	60	$C_{23}H_{20}N_4O_3$	C	69.00	69.04
						H	5.00	5.22
						N	14.00	14.30
III	H	4-NO_2	235°	76	$C_{23}H_{20}N_4O_3$	C	69.00	69.12
						H	5.00	5.08
						N	14.00	14.23
IV	H	2,4-$(NO_2)_2$	144°	68	$C_{23}H_{19}N_5O_5$	C	62.02	62.18
						H	4.27	4.31
						N	15.73	15.66
V	Cl	H	205°	80	$C_{23}H_{20}ClN_3O$	C	70.86	70.91
						H	5.13	5.22
						N	10.78	10.64
VI	Cl	2-NO_2	160°	63	$C_{23}H_{19}ClN_4O_3$	C	63.52	63.52
						H	4.37	4.34
						N	12.88	12.85
VII	Cl	4-NO_2	142°	78	$C_{23}H_{19}ClN_4O_3$	C	63.52	63.58
						H	4.37	4.41
						N	12.88	12.85
VIII	Cl	2,4-$(NO_2)_2$	165°	65	$C_{23}H_{18}ClN_5O_5$	C	57.56	57.53
						H	3.75	3.79
						N	14.60	14.68
IX	OCH_3	H	190°	80	$C_{24}H_{23}N_3O_2$	C	74.80	74.55
						H	5.97	5.89
						N	10.91	10.76
X	OCH_3	2-NO_2	156°	64	$C_{24}H_{22}N_4O_4$	C	66.98	66.92
						H	5.11	5.15
						N	13.02	13.27
XI	OCH_3	4-NO_2	250°	75	$C_{24}H_{22}N_4O_4$	C	66.98	66.89
						H	5.11	5.18
						N	13.02	13.00
XII	OCH_3	2,4-$(NO_2)_2$	140°	66	$C_{24}H_{21}N_5O_6$	C	60.63	60.68
						H	4.43	4.47
						N	14.73	14.55

[a] Melting points were taken in open capillary tubes and are corrected.

Male albino rats (100–150 g), kept on *ad libitum* diet, were used in all experiments: Rat brains isolated from decapitated animals were immediately homogenized in ice-cold 0.25 *M* sucrose in a homogenizer[3] in a ratio of 1:9 (w/v). All incubations were carried out at 37°, and the oxygen uptake was measured by the conventional Warburg manometric technique, using air as the gas phase (5).

Fresh brain homogenate, equivalent to 125 mg wet weight, was added to the chilled Warburg vessels containing 6.7 mmoles of magnesium sulfate, 20 mmoles of sodium hydrogen phosphate buffer solution (pH 7.4), 1 mmole of adenosine monophosphate (sodium salt), 33 mmoles of 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, β-hydroxybutyrate, and succinate were used at a final concentration of 10 m*M*, while the concentration of NADH was 0.5 m*M*. It was presumed that the endogenous NAD, present in the homogenate, was sufficient for these oxidative processes. All compounds under assay were dissolved in propylene glycol (100%), and an equal volume of the solvent was added to the control vessels.

RESULTS AND DISCUSSION

As evident from Table III, all pyrazolines possessed anticonvulsant activity and the degree of protection afforded by these compounds against pentylenetetrazol-induced convulsions ranged from 30 to 80%. Maximum protection was observed with compounds possessing 4-anisyl and 2,4-dinitrophenyl substituents at positions 1 and 5, respectively, of the pyrazoline nucleus (Compound XII). In general, pyrazolines possessing a 2,4-dinitrophenyl substituent at position 5 of the pyrazoline moiety (Compounds IV, VIII, and XII) possessed greater anticonvulsant activity. Introduction of a 4-chloro or 4-methoxy substituent on the phenyl nucleus attached to position 1 of the pyrazoline moiety (Compounds V–VIII and IX–XII) produced greater protection from pentylenetetrazol-induced seizures as compared to their respective unsubstituted 1-phenyl pyrazolines (Compounds I–IV).

Data on anticonvulsant activity of these pyrazolines and 24-hr pentylenetetrazol-induced mortality, in general, indicated a trend of some association between increased protection from convulsions and decreased pentylenetetrazol mortality in experimental animals. None of these pyrazolines exhibited any appreciable sedative or central nervous system-depressant effect or 24-hr mortality in a dose of 100 mg/kg as used in the present investigation.

All pyrazolines, except Compounds III, V, and IX, selectively inhibited NAD-dependent oxidation of pyruvate, α-ketoglutarate, β-hydroxybutyrate, and NADH by rat brain homogenates. On the other hand, NAD-independent oxidation of succinate remained unaltered. At present, it is difficult to explain the inability of Compounds III, V, and IX to affect NAD-dependent oxidations, and this property was not consistent with the nature of the substituents on the phenyl nuclei attached to positions 1 and 5 of the pyrazoline moiety. The ability of these pyrazolines to inhibit the oxidation of NADH provides evidence regarding the possible inactivation of the process of the electron transfer in the electron transport

[3] Potter–Elvehjem.

Table III—Anticonvulsant Activity and Inhibition of Respiratory Activity of Rat Brain Homogenate by 3-(3-Acetoamino)-phenyl-1,5-substituted Phenyl-Δ^2-pyrazolines

Compound	Anticonvulsant Activity[a], % Protection	Mortality[b] after 24 hr, %	Inhibition, %[c]			
			Pyruvate	α-Ketoglutarate	β-Hydroxybutyrate	NADH
I	30	50	37.0 ± 0.7	31.6 ± 0.6	46.5 ± 0.8	47.6 ± 0.6
II	40	30	59.0 ± 1.0	76.8 ± 0.8	61.6 ± 1.1	74.7 ± 0.8
III	30	60	Nil	Nil	Nil	Nil
IV	40	30	72.7 ± 0.5	75.3 ± 0.1	68.8 ± 1.1	69.9 ± 1.5
V	60	20	Nil	Nil	Nil	Nil
VI	60	Nil	70.7 ± 1.2	77.3 ± 0.9	84.2 ± 1.7	80.0 ± 1.8
VII	40	60	59.4 ± 0.9	61.1 ± 1.6	68.5 ± 0.3	56.2 ± 0.9
VIII	70	Nil	72.3 ± 1.3	75.3 ± 1.5	76.9 ± 0.9	62.3 ± 1.8
IX	40	40	Nil	Nil	Nil	Nil
X	50	20	39.3 ± 1.5	47.7 ± 1.2	66.8 ± 1.0	46.2 ± 1.2
XI	60	30	29.6 ± 0.9	14.5 ± 0.9	47.5 ± 1.1	34.8 ± 0.8
XII	80	10	50.2 ± 0.7	66.8 ± 1.3	79.8 ± 0.6	53.5 ± 0.7

[a] Screening procedures for the determination of anticonvulsant activity were described in the text. Substituted pyrazolines were administered at 100 mg/kg 4 hr before the administration of pentylenetetrazol. [b] Represents mortality during 24 hr in each group of animals administered pentylenetetrazol. [c] Each experiment was done in duplicate. All values represent mean values of percent inhibition with ± standard error of the mean from three separate experiments. Inhibition was determined by the decrease in the oxygen uptake/125 mg wet tissue weight/hr. The final concentrations of the various substrates, NADH, and substituted pyrazolines were 10, 0.5, and 1 m*M*, respectively. Vessel contents and assay procedures are described in the text.

chain by acting presumably at the site of transfer of electrons from NADH to flavine adenine dinucleotide. In the present study the selective inhibition of NAD-dependent oxidations by these pyrazolines was unrelated to their structure, so a definite structure–activity relationship was not exhibited (Table III).

These observations failed to provide any correlation between *in vitro* selective inhibition of NAD-dependent oxidations by these pyrazolines and their anticonvulsant activity. A detailed pharmacological and biochemical study using other purified enzyme systems may reflect the cellular basis for the anticonvulsant activity of these substituted pyrazolines.

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[x] To whom inquiries should be directed (at the University of North Dakota).