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Anticonvulsant effect of 5-ethyl, 5-phenyl, 2-pyrrolidinone and its possible relationship to γ-aminobutyric acid-dependent inhibitory mechanisms

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IN A PREVIOUS publication,[1] it was shown that 5-ethyl,5-phenyl,2-pyrrolidinone (EPP), a cyclic analog of γ-aminobutyric acid (GABA), is a potent anticonvulsant against convulsions induced by thiosemicarbazide, pentylenetetrazol (Metrazol) and electroshock, and has no effect on GABA metabolism. We now report the effects of EPP on the convulsions produced by other substances, which act apparently by blocking the physiological action of GABA. The results of these experiments permit an interpretation of the pharmacological effect of EPP.

Since EPP has some structural similarities with diphenylhydantoin (DPH) (Fig. 1), but some differences in anticonvulsant properties[1,2], it was considered of interest to study comparatively the action of these two drugs on the convulsant effect of bicuculline, a substance that acts most probably by blocking specifically the GABA receptors[3–5].

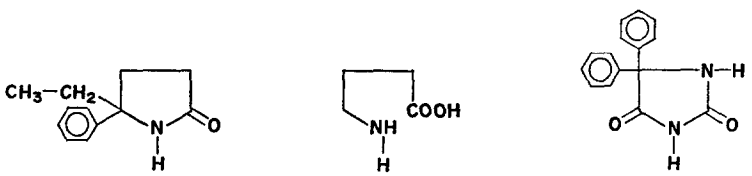

FIG. 1. Structures of EPP, GABA and DPH.

Adult mice from a local strain were used. The EPP was prepared by Carvajal *et al.*,[1] and the pyridoxal phosphate-γ-glutamyl hydrazone (PLPGH) was synthesized from pyridoxal-5′-phosphate and L-glutamic acid-γ-hydrazide, as previously described.[6] Other substances used were obtained from commercial sources [sodium diphenylhydantoin (Epamin) from Parke Davis, bicuculline from K & K Laboratories, Hollywood, Calif. and other chemicals from Sigma Chemical Co., St. Louis, Mo., or CalBiochem, La Jolla, Calif.]. All compounds, except bicuculline and DPH, were dissolved in water and injected intraperitoneally. Bicuculline was dissolved in diluted HCl (approx. 0·01 N) and the solution was adjusted slowly to pH 5–6 with NaOH. This drug was injected subcutaneously in the back of the mice. A more reproducible convulsant effect was obtained by this route of administration than after intraperitoneal injection. DPH was dissolved in a mixture of propyleneglycol and water (2:5 v/v), and injected intraperitoneally. Control animals were injected with 0·9% NaCl or with the DPH solvent. In all the experiments each mouse was handled individually and placed in a separate cage, to avoid the stimulatory effect of the convulsive activity of other animals.

Effects of EPP and DPH. When injected alone, EPP at a dose of 80 mg/kg produced only a slight decrease of motor activity. Ten to 20 min after a dose of 112 mg/kg an interesting sleeping-like condition was observed: the animals were standing on their hindlimbs, holding to the wires of the walls of the cage, and after some min they started to loose their paws until they "woke up" and held again firmly. This behavior was maintained for 20–60 min. The animals were hypotonic after a dose of EPP of 150 mg/kg or higher; they lost the righting reflex with 225 or 450 mg/kg (a complete recovery was observed after 80–120 min), and died in some minutes with 750 mg/kg, showing notable hypotonicity. DPH at a dose of 30 mg/kg produced no apparent effect up to 45 min after treatment. However, 120 mg/kg of DPH produced notable ataxia, which lasted for several hr. Mice chronically treated with DPH at 10 mg/kg/day or with EPP at the equimolar dose of 6·9 mg/kg/day, for 7 days, did not show any apparent behavioral alteration.

Simultaneous treatment with EPP (80 mg/kg) and DPH (30 mg/kg) resulted in a notable sedative or tranquilizer effect, clearly observed at 15 min after the injections; the mice were very quiet, and even partially lost the righting reflex. They, however, recovered completely after approximately 120 min.

Effects on convulsions. Treatment with EPP protected mice against the convulsions produced by Metrazol (the results of the experiments previously reported[1] were confirmed by using several doses of Metrazol), thiosemicarbazide, the simultaneous administration of L-glutamic acid-γ-hydrazide and pyridoxal phosphate, PLPGH and mercaptopropionic acid; EPP treatment decreased the number of animals convulsing, and/or significantly increased the time between the injection of the convulsant and the appearance of convulsions (Table 1).

The effect of EPP, DPH and EPP + DPH treatment on bicuculline-induced convulsions is shown in Table 2. Both compounds, when administered independently, exhibited clear anticonvulsant properties against bicuculline, but their mechanism of action seems to differ from one another. EPP decreased the percentage of mice showing tonic convulsion and the percentage of deaths, and significantly increased the time to tonic convulsions, whereas DPH completely prevented the tonic phase of convulsions, but most of the animals died. The simultaneous administration of EPP and DPH dramatically decreased both the number of animals convulsing and the number of deaths (Table 2). Treatment with EPP (6·9 mg/kg/day) or with DPH (10 mg/kg/day) for 7 days did not protect mice against the convulsions produced by a single dose of bicuculline (3 mg/kg) administered 24 hr after the seventh injection of EPP or DPH.

The effect of EPP on strychnine-induced convulsions was, surprisingly, opposite to that described for the other convulsant agents used in this work. EPP markedly potentiated the convulsant effect of this alkaloid (Table 3).

The pharmacological action of EPP could be explained postulating the following mechanism, which would account for both the anticonvulsant properties of this drug and its effect of potentiating the convulsant action of strychnine. We propose that EPP acts as a GABA-mimetic compound, inhibiting the neurons which are physiologically inhibited by GABA.[8] This hypothesis is based on: (a) the structural similarity of the EPP molecule (actually the product of hydrolysis of EPP, see Fig. 1 and Ref. 1) with GABA; (b) the finding that EPP exhibits notable anticonvulsant activity when the levels of GABA in brain are notably decreased by the simultaneous administration of glutamic acid-γ-hydrazide and pyridoxal phosphate,[7] without any changes in GABA metabolism due to EPP;[1,7] (c) the anticonvulsant action of EPP against bicuculline, which according to iontophoretical experi-

TABLE 1. EFFECT OF EPP ON CONVULSIONS INDUCED BY METRAZOL, THIOSEMICARBAZIDE, L-GLUTAMIC ACID-γ-HYDRAZIDE (GAH) + PYRIDOXAL PHOSPHATE (PLP), PLPGH AND MERCAPTOPROPIONIC ACID

Convulsant (dose)	EPP (dose)*	Mice showing tonic convulsions† (%)	Mean time to tonic convulsion (min)‡
Metrazol (75 mg/kg)	NaCl 0·9%	70 (10)	Not available (Ref. 1)
Metrazol (75 mg/kg)	EPP (80 mg/kg)	30 (10)	Not available (Ref. 1)
Thiosemicarbazide (20 mg/kg)	NaCl 0·9%	82·4 (34)	Not available (Ref. 1)
Thiosemicarbazide (20 mg/kg)	EPP (80 mg/kg)	30 (20)	Not available (Ref. 1)
GAH (80 mg/kg) + PLP (50 mg/kg)	NaCl 0·9%	100 (14)	38·3 [36–55]§
GAH (80 mg/kg) + PLP (50 mg/kg)	EPP (80 mg/kg)	20 (15)	47·5 [40–54]§
PLPGH (30 mg/kg)	NaCl 0·9%	54·2 (24)	47·5 [37–77]
PLPGH (30 mg/kg)	EPP (80 mg/kg)	60·0 (10)	71·0 [53–102]‖
PLPGH (50 mg/kg)	NaCl 0·9%	100 (10)	38·9 [29–47]
PLPGH (50 mg/kg)	EPP (100 mg/kg)	50 (10)	62·8 [49–81]‖
Mercaptopropionic acid (30 mg/kg)	NaCl 0·09%	60 (25)	5·7 [3–7]
Mercaptopropionic acid (30 mg/kg)	EPP (80 mg/kg)	10 (10)	7·0

* EPP was injected 30 min before the administration of Metrazol and mercaptopropionic acid, simultaneously to the administration of GAH + PLP and of PLPGH, and 30 min after thiosemicarbazide administration.

† Total number of mice in parentheses.

‡ If not indicated otherwise, mice died during tonic convulsions at the times indicated; range in brackets.

§ Data from Ref. 7.

‖ $P < 0·01$ (compared to control values). All animals that did not convulse died 60·3 min (mean time; range 47–71) after PLPGH injection.

TABLE 2. EFFECT OF EPP AND DPH ON CONVULSIONS INDUCED BY BICUCULLINE

Bicuculline dose (mg/kg)	Anticonvulsant (dose)*	Mice showing tonic convulsion† (%)	Mean time to tonic convulsion (min)‡
2	NaCl 0·9%	80 (5)	6·2 [3–12]
2	EPP (80 mg/kg)	20 (5)	7·0
2·5	NaCl 0·9%	85·7 (42)	9·1 [5–15]
2·5	EPP (80 mg/kg)	48·6 (35)	12·8 [6–25]
2·5	Solvent§	90 (10)	10·8 [5–17]
2·5	EPP in solvent (80 mg/kg)	90 (10)	15·3 [10–23]‖
2·5	DPH (120 mg/kg)¶	0 (10)	Seven mice died without tonic convulsion at 17·8 min [12–27]
2·5	EPP (80 mg/kg) + DPH (120 mg/kg)	0 (10)	One mouse died without tonic convulsion at 45 min
3	NaCl 0·9%	80 (20)	8·4 [6–13]
3	EPP (80 mg/kg)	55 (20)	9·9 [4–16]
3	Solvent	90 (10)	7·6 [4–11]
3	EPP in solvent (80 mg/kg)	60 (10)	19·8 [17–26]‖
3	DPH (30 mg/kg)	0 (10)	Seven mice died without tonic convulsion at 19 min [8–40]
3	EPP (80 mg/kg) + DPH (30 mg/kg)	0 (10)	Two mice died without tonic convulsion at 25 and 32 min

* EPP and DPH were injected 30 min before the administration of bicuculline.

† Total number of mice in parentheses.

‡ If not indicated otherwise, mice died during tonic convulsion at the times indicated; range in brackets.

§ Solvent for DPH: propyleneglycol–water (2:5, v/v).

‖ $P < 0{\cdot}01$ (compared to control values).

¶ Animals treated with this dose of DPH were ataxic.

ments is a specific blocking agent of the GABA receptor[3–5] (see, however, Refs. 9 and 10); (d) the protective action of EPP against PLPGH-induced convulsions, which are due to an inhibition of GABA synthesis at the synaptic endings[11–13]; and (e) the anticonvulsant effect of EPP against thiosemicarbazide and mercaptopropionic acid, which also produce convulsions probably as a consequence of glutamate decarboxylase inhibition.[14–17] The previously reported anticonvulsant effect of EPP against Metrazol and electroshock[1] could also be accounted for by a GABA-mimetic action of EPP, which would increase the threshold of the neurons directly depolarized by this type of convulsant agents. Although there is no direct neuropharmacological evidence for a GABA-like action of EPP, it is noteworthy that this compound was effective against convulsions induced by several substances with a mechanism of action most probably involving the metabolism or the action of GABA. Of particular importance are the effects of bicuculline and of PLPGH. Although not universally recognized,[9,10] the blocking action of bicuculline seems to be specific for GABA,[3–5] and all available evidence supports the conclusion that PLPGH-induced convulsions are due specifically to a decreased rate of synthesis of GABA at the nerve endings.[11–13,18] Furthermore, EPP, in contrast to many other anticonvulsant substances, prevents a wide variety of convulsions (electroshock, thiosemicarbazide, bicuculline, PLPGH, Metrazol and mercaptopropionic acid), with the only exception being strychnine-induced convulsions, which are potentiated by EPP. From this fact we may infer that the action of EPP probably involves a general inhibitory mechanism, like that dependent on GABA.

In order to explain the potentiation of strychnine-induced convulsions by EPP as opposed to its protective action against electroshock and the other convulsant drugs studied, we postulate that EPP acts as a GABA-mimetic compound at least at two different inhibitory neuronal systems. First, EPP would be inhibiting the motoneurons of the pyramidal cortex, and therefore blocking the convulsant action of electroshock, Metrazol, thiosemicarbazide, PLPGH, mercaptopropionic acid and bicuculline, as mentioned above. The inhibition of this system by GABA neurons is postulated to be a controlling

TABLE 3. EFFECT OF EPP ON CONVULSIONS INDUCED BY STRYCHNINE (BASE FORM)

Strychnine dose (mg/kg)	EPP* (dose)	Mice showing tonic convulsions† (%)	Mean time to tonic convulsion (min)‡
1·75	NaCl 0·9%	66·7 (15)	5·9 [3–7]
1·75	EPP (80 mg/kg)	100 (10)	5·4 [1–11]
1·25	NaCl 0·9%	10 (10)	5·0
1·25	EPP (80 mg/kg)	93·3 (15)	6·8 [4–18]

* EPP was injected 30 min before the administration of strychnine.
† Total number of mice in parentheses.
‡ All animals died during tonic convulsion at the times indicated; range in brackets.

mechanism continuously operating. Second, EPP would inhibit the neurons of the extrapyramidal cortex (mainly area 6), and possible other extrapyramidal nuclei, which under normal conditions are inhibiting spinal motoneurons.[19,20] Thus, the excitability threshold of the spinal motoneurons would be decreased by EPP. This hypothetical action of EPP would account for the potentiation of the convulsant effect of strychnine (Table 3), since the latter drug, especially at low doses, acts mainly by blocking the action of interneurons in the spinal cord (Renshaw cells), which use glycine as transmitter and inhibit the spinal motoneurons.[3,5,21] The possibility of such an action of EPP is supported by the fact that its administration to rats facilitates some spinal reflexes.[1] The above postulates are in accordance with the present physiological concepts of the pyramidal and extrapyramidal cortical systems.[19,20,22,23] Thus, our hypothesis of a GABA-mimetic action of EPP, admittedly based only on indirect, pharmacological evidence, provides a plausible explanation for the anticonvulsant properties of EPP as well as for its potentiating action on the convulsant effect of strychnine. Neurophysiological experiments on the effect of EPP on neuronal activity are necessary in order to substantiate or discard this hypothesis.

The mechanism of the anticonvulsant action of DPH appears to be different from that of EPP, since DPH is not effective against thiosemicarbazide[1] or Metrazol,[2] and its protective effect against bicuculline-induced convulsions is apparently of a different type as compared to EPP (see Table 2 and text above). Other factors, such as the binding of DPH to neuronal plasma membranes[24] and its effects on Na^+, K^+-ATPase,[25,26] on sodium influx,[27] or on the binding of catecholamines to nerve endings,[28] are possibly involved in the mechanism of the anticonvulsant effect of this hydantoin.

The tranquilizer effect of the simultaneous administration of EPP and DPH, as well as the notable additive anticonvulsant action of these drugs, makes this combination a potentially useful treatment for some psychic disorders and epilepsy.

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Regional brain salicylate concentrations in afebrile and febrile rabbitts

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THERE IS increasing evidence to indicate that inflammatory fevers are mediated by a circulating endogenous pyrogen (EP) which acts directly upon cells in the anterior hypothalamus[1] and midbrain.[2] This EP, which is a polypeptide of around 14,000 mol. wt,[3] can be released by leucocytes and other cells by a variety of stimuli including bacterial pyrogens.[4] It has been suggested that the action of EP within the brain is mediated by prostaglandins of the E series, in particular prostaglandin E_1[5].

Salicylates reduce the temperature of febrile (but not afebrile) animals by acting upon the same regions of the brain as EP.[6] Vane[7] has shown that salicylates will inhibit prostaglandin synthesis in guinea-pig lung homogenates and has suggested that the antipyretic action of the drug is produced by the same mechanism occurring within the central nervous system. Such a hypothesis depends, in part, on demonstrating that brain salicylate concentrations during antipyresis are sufficient to inhibit prostaglandin synthesis. The present study was performed in order to investigate this.

METHODS

Five afebrile and five febrile New Zealand white rabbits weighing 2–3 kg were used in these experiments. They were restrained in conventional headstocks and temperature was measured with rectal thermistors advanced 8–10 cm. Fever was induced by an intravenous priming injection (2 ml) followed by a sustaining infusion for 4 hr of homologous plasma containing EP,[8] which caused a rise in temperature of 1·0–1·5° over the first hour. Thereafter temperature remained stable for as long as the EP infusion continued. Both febrile and afebrile animals each received 50 mg/kg ^{14}C-salicylate (carboxylic label, Radiochemical Centre, Amersham, England) at an approximate specific activity