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Stimulation of Gastric Mucosal Afferents with Mesaton Potentiates the Anticonvulsant and Eliminates the Sedative Actions of Sodium Valproate in Rats with Corasol Kindling

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Chronic oral sodium valproate given at high doses (100–200 mg/kg) suppressed the development of generalized clonic-tonic corasol (pentylenetetrazole) kindled convulsions in 100% of rats but prevented local clonic kindled convulsions in only 33–57%. At these doses, sodium valproate induced strong sedation. Combined chronic oral administration of Mesaton at the threshold dose of 0.2 mg/kg, inactive when given alone, and sodium valproate at the high doses of 100 and 200 mg/kg potentiated the anticonvulsant action of sodium valproate and prevented not only clonic-tonic kindled convulsions in 100% of rats, but also clonic kindled convulsions in 86–100% of rats, and also increased the anticonvulsant activity of valproate by factors of 1.7–1.9. These combinations of sodium valproate with Mesaton did not induce any sedative side effect. The mechanism of potentiation of the anticonvulsant effect and elimination of the sedative side effect of high-dose sodium valproate is based on stimulation of gastric mucosal afferents by Mesaton.

Keywords: valproate, Mesaton (pentylenetetrazole), kindling, convulsions, sedation, afferents, vagus.

Corasol (pentylenetetrazole) kindling is a widely used model of chronic temporal lobe epilepsy in rats, which is used to study the effects of agents on epileptogenesis and the generation of convulsions during the development of kindling [8, 9, 18].

sodium valproate is a standard antiepileptic agent, which provides the most effective suppression of the development of generalized clinic-tonic corasol kindled convulsions in chronic prophylactic administration in rats and mice, although it prevents local clonic kindled convulsions in only some proportion of the animals [3, 11]. A disadvantage of sodium valproate is its powerful sedative action at high therapeutic doses [2, 5, 12–15].

Threshold doses of adrenaline are known to potentiate the anticonvulsant actions of diazepam at low and intermediate doses in a model of acute corasol convulsions, without any increase in its sedative activity in an open field test [1]. We suggested that the adrenomimetic Mesaton, used at a threshold dose, which when used along is ineffective, would potentiate the anticonvulsant actions and eliminate the sedative side effect of sodium valproate at high therapeutic doses when given as combined chronic oral administration in a model of corasol kindling in rats.

The aim of the present work was to study the effects of a threshold dose of Mesaton on the anticonvulsant and sedative effects of sodium valproate.

Methods

Experiments were performed on white male Wistar rats weighing 180–200 g. The animals' sensitivity to single i.p. doses of corasol at the minimum active dose of 60 mg/kg was tested on experimental day 1. Further investigations were performed on selected rats which developed clonic and clonic-tonic convulsions (severity 2–4 points) within 30 min of administration of the corasol test dose.

Corasol kindling was developed by giving the selected rats i.p. corasol at the subconvulsive dose of 40 mg/kg ev-

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ery other day from experimental day 3 to experimental day 21. Animals were monitored and their behavior recorded for 30 min after each dose of corasol. Signs of convulsive activity were evaluated on the Racine scale: 0 corresponded to the absence of any reaction; 1 to facial automatisms, pricking up of the ears and whiskers; 2 to convulsive waves propagating along the axis of the trunk; 3 to myoclonic convulsions with rising; 4 to clonic convulsions with lose of posture; 5 to repeated powerful clonic-tone convulsions; 6 to tonic convulsions and the animal's death [18]. Convulsions of stages 1–3 were local clonic convulsions, while those of 4–5 were generalized clonic-tonic convulsions.

Completely kindled rats demonstrated at least three sequential stage 4–5 generalized convulsions to the last three doses of corasol. Rats were regarded as tolerant to kindling (non-kindled rats) if they showed convulsions below stage 2.

The effects of substances on the epileptogenesis of kindled convulsions and the development of complete kindling were studied using a prophylactic regime, in which substances were given chronically before each injection of the subconvulsive dose of corasol from experimental day 3 to experimental day 21 [8, 9, 18].

Experimental groups:

1. Control (distilled water, intragastric) – eight rats.
2. Sodium valproate 100 mg/kg, intragastric – seven rats.
3. Sodium valproate 200 mg/kg, intragastric – seven rats.
4. Mesaton 0.2 mg/kg, intragastric – seven rats.
5. Mesaton 1.0 mg/kg, intragastric – seven rats.
6. Sodium valproate 100 mg/kg + Mesaton 0.2 mg/kg, intragastric – seven rats.
7. Sodium valproate 200 mg/kg + Mesaton 0.2 mg/kg, intragastric – eight rats.
8. Sodium valproate 100 mg/kg + Mesaton 0.2 mg/kg + lidocaine 1%, 1 ml, intragastric – seven rats.
9. Sodium valproate 200 mg/kg + Mesaton 0.2 mg/kg + lidocaine 1%, 1 ml, intragastric – eight rats.

Mesaton (Sigma) at doses of 0.2 and 1.0 mg/kg, sodium valproate (Sigma) at doses of 100 and 200 mg/kg, and combinations of valproate at doses of 100 and 200 mg/kg with Mesaton at the threshold dose of 0.2 mg/kg were given daily via the oral intragastric route using rigid metal probes 45 min before administration of corasol from experimental day 3 to experimental day 21. Animals of the control group received 1 ml of distilled water via the intragastric route 45 min before corasol on experimental day 3 to experimental day 21.

Throughout the experiment, mean convulsion severity was assessed in points for each dose of study substances and controls (groups of 7–8 rats), along with the number and proportion of completely kindled rats and the number and proportion of rats without kindling (convulsion severity of less than 2 points).

The anticonvulsant actions of sodium valproate, Mesaton, and combinations of sodium valproate with Mesaton were evaluated quantitatively on kindling day 21, i.e., after completion of substance administration using the prophylactic regime, in terms of reductions in the mean severity of kindled convulsions as compared with controls, and also in terms of the reduction in the number of completely kindled rats and the increase in the number of rats without kindling as a proportion in comparison with the control group.

The sedative actions of substances were evaluated using an open field (OF) test [1, 4]. The OF test was used to determine the rats' locomotor activity. The animal was placed in the center of a square illuminated field (1 × 1 m) and the total time spent walking was measured in seconds (horizontal activity), and the number of rearings onto the hindlimbs was also counted (vertical activity). Horizontal and vertical motor activity was measured in kindled rats on experimental day 21, 40 min after the last dose of study substance and 5 min before administration of corasol. Locomotor activity was quantitated for each dose of study substances in terms of mean horizontal and vertical activity. The sedative actions of sodium valproate, Mesaton, and combinations of sodium valproate with Mesaton at the threshold dose were assessed in terms of reductions in mean horizontal and vertical activity in the OF test as percentages of values in control group animals on experimental day 21.

The role of gastric mucosal afferents in the mechanism of Mesaton potentiation of the anticonvulsant action and elimination of the sedative side effect of sodium valproate was studied by intragastric administration of 1% lidocaine solution (1.0 ml) 30 min before the combination of sodium valproate (100 and 200 mg/kg) and Mesaton (0.2 mg/kg) on experimental days 3 to 21.

Experimental data from the corasol kindling model were analyzed statistically using Fisher's test (frequency of kindling convulsions) and the Mann–Whitney test (severity of kindled convulsions). Data from OF test experiments were analyzed statistically using Student's *t* test.

Results and Discussion

Corasol kindling was modeled by repeatedly giving rats corasol at a dose not inducing convulsive states. This intervention produced a gradual increase in the convulsive "readiness" of the brain to the action of the convulsant. This was apparent in that the previously inactive subconvulsive dose of corasol induced convulsions of increasing severity, ending with generalized clonic-tonic seizures in kindled rats [3, 8, 21].

As shown in Table 1, repeat (from injection 7 to injection 11) i.p. doses of corasol at the subconvulsive dose of 40 mg/kg to rats of the control group (daily intragastric administration of distilled water 45 min before corasol) led to the development of kindled convulsions of severity 3–5 points by experimental day 21 in all eight rats, while complete kindling (repeated generalized and clonic-tonic convulsions of severity 4–5 points) occurred in six of the eight rats (75% completely kindled rats). The mean severity of kindled convulsions in rats of the control group on experimental day 21 was 3.8 ± 0.9 points.

Rats of the control group were highly active in the open field test 5 min before corasol on experimental day 21

TABLE 1. Effects of Sodium Valproate and Mesaton on the Development of Corasol Kindling and the Severity of Kindled Convulsions in Rats

Substance	Dose (intragastric),* mg/kg	Total number of rats in group	Number of fully kindled rats,** (% of total number of rats in group)	Number of rats without kindling,*** (% of total number of rats in group)	Mean severity of kindled convulsions, points****
Control (distilled water)	—	8	6 (75 %)	0 (0 %)	3.8 ± 0.9
Sodium valproate	100	7	1 (14 %)[1]	2 (29 %)	2.6 ± 0.6
	200	7	0 (0 %)[1]	4 (57 %)[2]	2.0 ± 0.5[2]
Mesaton	0.2	7	4 (57 %)	0 (0 %)	3.5 ± 0.7
	1.0	7	2 (29 %)[2]	0 (0 %)	2.8 ± 0.4
Sodium valproate + Mesaton	100 + 0.2	7	0 (0 %)[1]	6 (86 %)[1]	1.4 ± 0.3[2]
Sodium valproate + Mesaton	200 + 0.2	8	0 (0 %)[1]	8 (100 %)[1]	1.2 ± 0.3[2]
Sodium valproate + Mesaton + 1 ml of 1% lidocaine*****	100 + 0.2	7	1 (14 %)[1]	2 (29 %)	2.7 ± 0.5
Sodium valproate + Mesaton + 1 ml of 1% lidocaine*****	200 + 0.2	8	0 (0 %)[1]	4 (50 %)[2]	2.2 ± 0.3[2]

* At a dose of 40 mg/kg 45 min before corasol from day 3 to day 21. ** Generalized clonic-tonic convulsions of severity 4–5 points after the last three doses of corasol at the subconvulsive dose of 40 mg/kg, i.p. *** Clonic convulsions of severity less than 2 points on kindling day 21. **** On kindling day 21. ***** 30 min before administration of the combination of sodium valproate and Mesaton from day 3 to day 21. [1] $p < 0.01$, [2] $p < 0.05$ compared with controls.

TABLE 2. Effects of Sodium Valproate, Mesaton, and Their Combinations on the Behavior of Rats in the Open Field Test*

Substance	Dose (intragastric),** mg/kg	Total number of rats in group	Mean horizontal activity, sec	Mean vertical activity, rearings
Control (distilled water)	—	8	15.6 ± 1.8	5.5 ± 0.6
Sodium valproate	100	7	3.6 ± 0.7[2]	1.2 ± 0.3[2]
	200	7	2.5 ± 0.5[1]	0.7 ± 0.2[1]
Mesaton	0.2	7	15.3 ± 1.6	5.3 ± 0.6
	1.0	7	17.4 ± 1.9	5.8 ± 0.9
Sodium valproate + Mesaton	100 + 0.2	7	13.8 ± 2.0	5.0 ± 0.7
Sodium valproate + Mesaton	200 + 0.2	8	11.9 ± 1.8	4.4 ± 0.7
Sodium valproate + Mesaton + 1 ml of 1% lidocaine***	100 + 0.2	7	4.0 ± 0.7[2]	1.4 ± 0.3[2]
Sodium valproate + Mesaton + 1 ml of 1% lidocaine***	200 + 0.2	8	2.9 ± 0.6[1]	0.9 ± 0.3[1]

* Kindling day 21. ** 45 min before administration of corasol at a dose of 40 mg/kg from day 3 to day 21. *** 30 min before administration of the combination of sodium valproate with Mesaton from day 3 to day 21. [1] $p < 0.01$, [2] $p < 0.05$ compared with controls.

(mean horizontal activity 15.6 ± 1.8 sec, mean vertical activity 5.5 ± 0.6 rearings).

Sodium valproate is the standard antiepileptic substance, which when given as chronic prophylactic treatment in rats and mice produces a maximal level of suppression of the development of generalized clonic-tonic corasol kindled convulsions, though local clonic kindled convulsions are prevented in only a proportion of the animals [3, 11].

In the present experiments, chronic oral administration of prophylactic sodium valproate at the high doses of 100 and 200 mg/kg decreased the mean severity of kindled convulsions by factors of 1.5–1.9 on kindling day 21 ($p < 0.05$, Table 1), and also decreased the proportions of completely kindled rats by 86% and 100% from the control level ($p < < 0.01$, Table 1). At a dose of 200 mg/kg, sodium valproate significantly (by 57%) decreased the number of rats without kindling, i.e., having convulsion severity of less than 2 points, from the control level ($p < 0.05$, Table 1).

The results obtained here provided evidence that sodium valproate at the high dose of 200 mg/kg given by an oral

prophylactic regime produced the maximal suppression of the development of generalized clonic-tonic kindled convulsions of severity 4–5 points (in 100% of rats), though the development of local clonic kindled convulsions of severity 2–3 points occurred in only 57% of rats.

The anticonvulsant action of sodium valproate is known to develop only at high doses of 100–200 mg/kg, which induce a sedative side effect, apparent as significant reductions in horizontal and vertical movement activity in the OF test [14, 19]. As shown in Table 2, oral administration of sodium valproate to kindled rats at the high doses of 100 and 200 mg/kg decreased horizontal activity on experimental day 21 by factors of 4.3 and 6.9, respectively, from the control level, with decreases in vertical activity by factors of 4.6 and 7.9. Thus, the results of these experiments demonstrate the existence of a powerful sedative effect of high doses of sodium valproate in the OF test on the last day of kindling.

Chronic oral administration of the adrenomimetic Mesaton using a prophylactic regime at a threshold dose of 0.2 mg/kg on experimental day 21 produced a minor and insignificant decrease in the number of fully kindled rats, with essentially no decrease in the mean severity of kindled convulsions from the control level (Table 1). Chronic oral administration of a higher dose of Mesaton, 1 mg/kg, decreased the number of fully kindled rats on experimental day 21 by 59% ($p < 0.05$), though the decrease in the mean severity of kindled convulsions was not significantly decreased compared with controls (Table 1). In contrast to sodium valproate, neither dose of Mesaton had any sedative side effect, as there were no changes in motor activity in the OF test (Table 2).

Combined chronic oral administration of sodium valproate at the high doses of 100 and 200 mg/kg with Mesaton at the threshold dose of 0.2 mg/kg produced the maximum anticonvulsant effect, which could not be achieved by administration of valproate alone, on experimental day 21, with suppression of the development not only of generalized clonic-tonic corasol kindled convulsions in 100% of rats, but also of clonic kindled convulsions in 86–100% of rats ($p < 0.01$, Table 1); the mean severity of corasol kindled convulsions, which were 1.9 and 1.7 times greater than obtained with sodium valproate given alone, decreased ($p < 0.05$, Table 1).

As shown in Table 2, oral administration of sodium valproate at the intermediate dose of 100 mg/kg and the high dose of 200 mg/kg in combination with Mesaton at the threshold dose of 0.2 mg/kg produced virtually no sedative effect on experimental day 21, as horizontal and vertical activity in the OF test decreased by only 10–20% from the control levels, as compared with reductions by 84–87% obtained with sodium valproate alone (Table 2). Thus, Mesaton in combination with sodium valproate eliminates the sedative action of sodium valproate in rats with corasol kindling.

Prior anesthesia of the gastric mucosa with 1% lidocaine solution in kindled rats on experiments days 3–21 completely eliminated the Mesaton potentiation of the anticonvulsant action of sodium valproate at both the intermediate dose of 100 mg/kg and the high dose of 200 mg/kg in combination with Mesaton at the threshold dose of 0.2 mg/kg, as the anticonvulsant activity of these combinations decreased to the initial anticonvulsant activity of sodium valproate alone (Table 1). Anesthesia of the gastric mucosa with 1% lidocaine solution in kindled rats from experimental day 3 to experimental day 21 almost completely restored the sedative action of sodium valproate at both doses on kindling day 21 despite being combined with Mesaton (Table 2).

It follows from here that stimulation of gastric mucosal afferents with Mesaton not only potentiated the anticonvulsant action, but also eliminated the sedative side effect of sodium valproate at high doses in rats with corasol kindling.

Stimulation of gastric vagal afferents by a threshold dose of adrenaline is known to potentiate the anticonvulsant action of diazepam at low and intermediate doses when given as single combined intramuscular doses in a model of acute corasol convulsions without increasing the sedative effect of diazepam in the open field test [1]. We suggested that the adrenomimetic Mesaton, like adrenaline, when given by chronic oral administration, would also potentiate the anticonvulsant effect and simultaneously eliminate the sedative side effect of sodium valproate at high therapeutic doses due to stimulation of gastric mucosal vagal afferents in rats with corasol kindling.

Valproate has been shown to suppress epileptogenesis of generalized kindled convulsions by increasing GABAergic inhibition of pyramidal neurons in the hippocampus and cortex, induced by inhibition of GABA transaminase, and increased release of GABA from terminals. However, valproate had no significant effect on the epileptogenesis of clonic kindled convulsions, as it did not weaken the toxic effect of glutamate in kindling [6, 7, 20].

Electrical stimulation of the vagus is known to prevent the degeneration of hippocampal neurons provoked by cerebral ischemia by suppressing the release of endogenous glutamate and weakening of the excitotoxic action of glutamate after ischemia [10]. It can be suggested that that Mesaton potentiation of the anticonvulsant action of valproate in rats with corasol kindling is based on a decrease in the toxic action of glutamate on hippocampal and cortical neurons induced by stimulation of the afferent vagus by Mesaton.

Known antiepileptic substances are insufficiently effective against the clonic phase of convulsions in patients with temporal lobe epilepsy, even at high doses inducing the sedative side effect [5, 7, 16, 17]. The results obtained here provide evidence that the combined chronic oral administration of sodium valproate at a high therapeutic dose with Mesaton at a threshold dose provided the maximum level of suppression of epileptogenesis not only in relation to clon-

ic-tonic but also in relation to local clonic kindled convulsions, also eliminating the sedative side effect of sodium valproate.

We suggest that the combined use of a threshold dose of Mesaton with sodium valproate and other antiepileptic substances at high therapeutic doses provides a more effective and safer approach to the treatment of antiepileptic-resistant temporal lobe epilepsy.

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