The Effect of Altered Reticuloendothelial Function on Electroshock Seizure Pattern

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INTRODUCTION

It is well known that various endocrinological interventions affect brain excitability and reticuloendothelial (RE) function.[3)7)8)14)16)] Interestingly, the direction and intensity of the change in the seizure threshold produced by such procedures are generally the same as those in RE function. For example, adrenalectomy and cortisone markedly decrease, ACTH moderately increases and corticosterone does not alter, both the seizure threshold and RE function. This fact seems to suggest the possibility that RE function may have some relation to brain excitability. Thus, the effect of altered RE function on brain excitability was studied experimentally. This paper deals with changes in electroshock seizure pattern produced by RES depressants and stimulants. The effect on the electroshock seizure threshold will be described later.

MATERIAL AND METHODS

Animals. Experiments were performed on male dd mice, with an initial body weight of 20 to 25 g, in room temperature. The animals were maintained on Oriental Chow and water.

Drugs. Perikan ink[1)] (Günther Wagner) and methyl palmitate[5)] (Katayama Kagaku Kōgyō) were used as RES depressants, while zymosan[10)] (Kyokasei Kōgyō) and diethylstilbesterol[7)] (Nakarai Chemicals) were used as RES stimulants. The doses, routes and periods of administration were as follows: Perikan ink, 5 ml/kg of 3 % solution, intraperitoneally, 3 and 7 days; methyl palmitate, 800 mg/kg, intraperitoneally, 3 days; zymosan, 40 mg/kg, subcutaneously or intraperitoneally, 3 days; and diethylstilbesterol, 10 and 20 mg/kg, subcutaneously, 3 days. Methyl palmitate was suspended in 0.1 % Tween 20 in Ringer's solution and the other drugs were dissolved in Ringer's solution. The solution containing diethylstilbesterol was adjusted to pH 10 by adding NaOH, in order to increase its solubility. All drugs were administered once a day. The injection volume of the solution

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was 0.05 ml except for Perikan ink. In order to block the RES, 5ml/kg of 3 % Perikan ink was intraperitoneally injected for 10 days. Subsequently, the RES stimulants were administered for 3 days. The experiments were performed 24 hours after the last injection.

Adrenalectomy. Adrenalectomy was done under ether anesthesia by a retroperitoneal approach. Adrenalectomized mice received 0.9 % NaCl solution in drinking water and a daily subcutaneous dose of 2 μg of dexamethasone phosphate (Decadron). The experiments were started on the day following adrenalectomy and the RES stimulants were administered daily for 3 consecutive days.

Electroshock seizure pattern. Maximal electroshock seizures were induced by Woodbury and Davenport's apparatus.[15] As electrical stimulus, 60 cycle/sec alternating current of 40 mA was applied by means of corneal electrodes for 0.2 sec. The maximal seizures are consisted of tonic hindlimb flexion (TF), tonic hindlimb extension (TE) and clonus. The duration of each phase was timed to the nearest 0.1 sec.

Acid phosphatase. Liver specimens were fixed in cold aceton and paraffin sections were used for histochemical detection of acid phosphatase. The histochemical method used was the lead nitrate method after Gomori.[9] In each experimental group, the results were expressed as changes from the controls.

RESULTS

The effect of RES depressants on electroshock seizure pattern

Changes in seizure pattern produced by Perikan ink and methyl palmitate are shown in Table 1. The duration of clonus is not presented, since it was not significantly changed in each experiment. Both Perikan ink and methyl palmitate prolonged TF and shortened TE. Since the prolongation of TF produced by Perikan ink occurred as early as 3 days and methyl palmitate prolonged TF highly significantly, it seems that RES depressants affect TF more than TE.

Table 1. Effect of RES depressants on electroshock seizure pattern

Treatment	No. of mice	TF	TE	
		sec	sec	% change
Control	10	1.1 ± 0.21	11.0 ± 1.2	
Perikan ink 3 % 5ml/kg, ip, 3d	10	1.3 ± 0.14*	10.9 ± 1.3	− 1
Control	10	1.4 ± 0.23	12.8 ± 1.5	
Perikan ink 3 % 5ml/kg, ip, 7d	12	1.8 ± 0.38*	10.2 ± 2.0**	−20
Control	18	1.2 ± 0.18	12.8 ± 1.6	
Methyl palmitate 800mg/kg, ip, 3d	15	1.4 ± 0.21**	10.6 ± 1.3*	−17

Each value represents mean ± S.D.
* P <0.05
**P <0.01
TF: Tonic flexion phase
TE: Tonic extension phase

The effect of RES stimulants on electroshock seizure pattern

Changes in seizure pattern produced by zymosan and diethylstilbesterol in intact and adrenalectomized mice are shown in Table 2. On subcutaneous administration, zymosan only slightly increased TE. However, intraperitoneal injection of the same dose of zymosan significantly shortened TF and prolonged TE. Diethylstilbesterol prolonged TE and shortened TF in a dose of 20 mg/kg. Such prolongation of TE produced by the RES stimulants was more pronaunced in adrenalectomized mice; changes in TE produced by subcutaneous injection of zymosan and diethylstilbesterol (10 mg/kg) were +20 and +19 % respectively, being about three times and twice as great as in intact mice.

Table 2. Effect of RES stimulants on electroshock seizure pattern

Treatment	No. of mice	TF	TE		Increase in acid phosphatase activity of Kupffer's cells
		sec	sec	% change	
Intact					
Control	11	1.4±0.24	10.4±0.9		
Zymosan 40mg/kg sc	9	1.3±0.20	11.0±0.9	+ 6	slight or moderate
Diethylstilbesterol 10mg/kg sc	11	1.3±0.23	11.4±0.9*	+10	slight or moderate
Control	10	1.7±0.50	10.6±1.6		
Zymosan 40mg/kg ip	15	1.2±0.33**	12.1±1.7*	+14	
Control	8	1.3±0.16	10.6±1.7		
Diethylstilbesterol 20mg/kg sc	8	1.1±0.15*	13.1±2.3*	+24	moderate or marked
Adrenalectomized					
Control	15	1.4±0.28	10.8±1.4		
Zymosan 40mg/kg sc	10	1.3±0.30	13.0±2.6*	+20	moderate
Diethylstilbesterol 10mg/kg sc	12	1.3±0.25	12.9±1.9*	+19	moderate

Each value represents mean ± S. D.
* $P<0.05$
** $P<0.01$
TF: Tonic flexion phase
TE: Tonic extension phase

Acid phosphatase activity of Kupffer's cells in the liver was increased in intact and adrenalectomized mice. The increase in the enzymatic activity was more prominent in adrenalectomized mice.

The effect of RES stimulants on electroshock seizure pattern in RES blocked mice

As shown in Table 3, zymosan and diethylstilbesterol had little or no effect on seizure pattern in RES blocked mice. This was true in both intact and adrenalectomized mice. These findings indicate that the observed prolongation of TE produced by the RES stimulants largely disappeared following the blockade of the RES.

In intact and adrenalectomized mice, acid phosphatase activity of Kupffer's

cells was slightly increased following the administration of zymosan and diethylstilbesterol.

Table 3. Effect of RES stimulants on electroshock seizure pattern in RES blocked mice

Treatment	No. of mice	TF	TE		Increase in acid phosphatase activity of Kupffer's cells
		sec	sec	% change	
Intact					
Control	8	1.5±0.13	10.4±1.7		
Zymosan 40mg/kg sc	8	1.5±0.36	10.4±1.8	0	slight
Diethylstilbesterol 10mg/kg sc	10	1.4±0.11	11.0±1.8	+9	slight or no
Adrenalectomized					
Control	9	1.5±0.36	10.8±1.7		
Zymosan 40mg/kg sc	9	1.5±0.31	11.4±1.1	+6	slight
Diethylstilbesterol 10mg/kg sc	9	1.5±0.29	11.7±1.1	+8	slight

Each value represents mean ± S. D.

TF: Tonic flexion phase
TE: Tonic extension phase

DISCUSSION

The present study showed that the RES depressants and stimulants produced changes in electroshock seizure pattern; Perikan ink and methyl palmitate prolonged TF and shortened TE while zymosan and diethylstilbesterol prolonged TE and tended to shorten TF. Since longer TF and/or shorter TE indicate decreased brain excitability and the reverse is true for increased brain excitability,[2)6)] it may be said that the RES depressants decreased brain excitability while the RES stimulants increased it.

DiCarlo et al.[3)4)] and Wexler[12)13)] have shown that RES stimulants likewise act on the adrenal cortex in intact animals, presumably by releasing ACTH. Therefore, it would be possible that the observed changes in seizure pattern produced by the RES stimulants are caused by the action on the adrenal cortex, which has an intimate relation to brain excitability. However, this possibility can be excluded by the present observation that the prolongation of TE produced by the RES stimulants was also observed in adrenalectomized mice.

It should be noted here that the prolongation of TE following the administration of the RES stimulants was more pronaunced in adrenalectomized mice.

Based on the view that RES stimulants interact physiologically with adrenocortical hormons,[3)4)] functional unbalance in the endocrine system and the RES following adrenalectomy might account for this phenomenon.

On the other hand, the blockade of the RES with Perikan ink decreased the prolongation of TE produced by the RES stimulants in both intact and adrenalectomized mice. In this case, acid phosphatase activity of Kupffer's

cells in the liver was only slightly increased by the RES stimulants, in contrast with a moderate increase in the mice whose RES was not blocked by Perikan ink. Since acid phosphatase activity of Kupffer's cells principally represents the RE activity,[11] it may safely be said that the decrease in the prolongation of TE in RES blocked mice was caused by a decrease in RE function and, in other wards, the prolongation of TE produced by the RES stimulants was at least mostly due to increased RE function.

From the present experimental findings and the foregoing discussion, it may be concluded that altered RE function produces changes in electroshock seizure pattern ; i. e., decreased RE function prolongs TF and shortens TE and the reverse is true.

SUMMARY

The effect of altered RE function on electroshock seizure pattern was studied in mice. RES depressants (Perikan ink and methyl palmitate) produced the prolongation of TF and shortening of TE (decrease in brain excitability), while RES stimulants (zymosan and diethylstilbesterol) produced the prolongation of TE and shortening of TF (increased brain excitability). The effect of the RES stimulants on TE was more pronaunced in adrenalectomized mice and was largely abolished in RES blocked mice. This indicates that the prolongation of TE produced by the RES stimulants was not mediated by the adrenal gland and was at least mostly caused by increased RE function.

From these observations, it is reasonable to conclude that altered RE function produces changes in electroshock seizure pattern.

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