ANALYSIS OF THE COMPOSITION OF THE WAVE OF THE DORSAL SURFACE POTENTIAL OF THE SPINAL CORD IN RATS WITH ASCENDING TETANUS AND A PAROXYSMAL SYNDROME

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UDC 616.981.551-031.67-092.9-06: 616.8-009.24-07: 616.832-073.97

The dorsal surface potentials and efferent discharge (ED) were investigated in the ventral roots of the spinal cord of healthy rats, rats with local tetanus, and rats with ascending tetanus associated with a paroxysmal syndrome. In rats with ascending tetanus a considerable increase in amplitude and duration of the positive dorsal surface potential (the P wave) produced by stimulation of a nerve on the side of the tetanus was observed. By using experimental procedures shortening or lengthening the ED (injection of nembutal, asphyxia, application of double or repetitive stimulation of nerves), the presence of a component which changed under different experimental conditions in the same way as the paroxysmal ED (the "paroxysmal" component) was demonstrated in the P wave of rats with ascending tetanus. The suggestion is made that the P wave produced by stimulation of a nerve on the "tetanus" side in rats with a paroxysmal syndrome associated with ascending tetanus is heterogeneous and contains two components: a component analogous to the P wave in the healthy animal and a "paroxysmal" component.

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It was shown previously [3, 4] in rats with ascending tetanus that stimulation of nerves of the limb into which the toxin was injected, producing generalized spasms (the phenomenon of the "universal departure station" [2]) leads to the appearance of dorsal root potentials of increased amplitude and duration, and to a corresponding positive dorsal surface potential of the spinal cord (a P wave), and that the durations of the augmented P wave and the efferent paroxysmal discharge are identical.

Since the efferent discharge (ED) and the P wave are generated by different structures and reflect different central processes [5], their coincidence in time could indicate the presence of a component in the P wave of rats with ascending tetanus which differs in its properties from the P wave of the healthy rats. Data supporting this suggestion are given below.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 300-500 g. Local tetanus was produced by injection of tetanus toxin in a dose of 0.05 MLD, and ascending tetanus by injection of a dose of 6 MLD into the gastrocnemius muscle. In the latter case spread of the toxin from the blood was blocked by injection of tetanus antiserum in a dose of 0.025 antitoxin unit. MLD was determined in rats weighing 200-220 g. Potentials were recorded on the 5th day after injection of the toxin. The previous day the spinal cord of all the animals was divided at the level T_8. On the day of the experiment, under ether anesthesia tracheotomy and laminectomy (L_1-S_1) were performed and the roots and nerves were dissected. Ventral roots (L_4, L_5, L_6, S_1) were identified and divided on both sides. The sural, peroneal, and tibial nerves were dissected and divided distally in both limbs, as well as all muscular branches of the sciatic nerve in the thigh. Curarization (0.2-0.4 mg/kg) and artificial respiration were applied. The active electrode (a thin platinum wire) used to record the dorsal surface potentials was placed in the midline of the spinal cord at the level L_6. The reference electrode was fixed to the spinal muscles. The ventral root potentials were recorded with bipolar electrodes by the usual method. The potentials were fed into an ac amplifier (time constant about 2 sec) and photographed from the screen of a dual-beam CRO. The nerves were stimulated by

Laboratory of Infectious Pathology, Institute of Normal and Pathological Physiology, Moscow (Presented by Academician V. V. Parin). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 4, pp. 33-38, April, 1969. Original article submitted August 2, 1968.

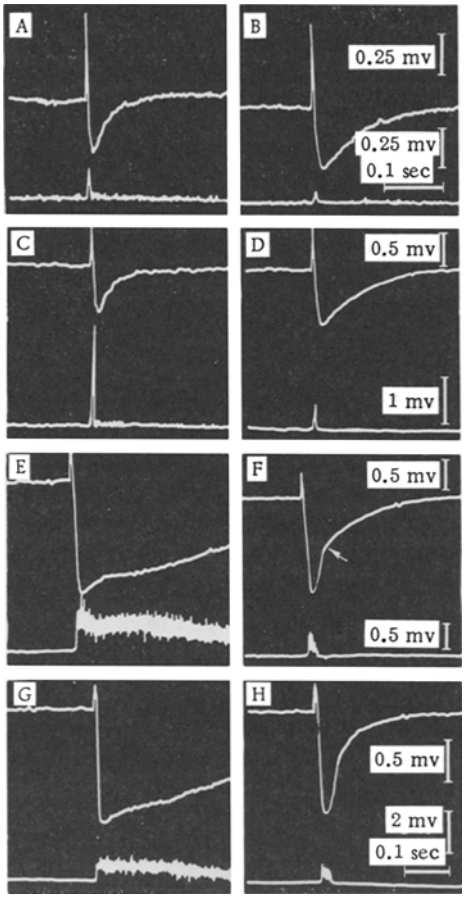

Fig. 1. Action of nembutal on dorsal surface potentials (DSPs) and efferent discharges (EDs) in ventral roots of rat spinal cord. In all curves, DSPs are shown above and EDs below. Strength of stimulation of nerves for all traces identical, 40T. A and B) DSPs and EDs of root L_5 in a healthy rat; C and D) DSPs and EDs of L_5 evoked by stimulation of sural nerve on "tetanus" side in rats with ascending tetanus; A, C, E, G before; B, D, F after injection of nembutal in dose of 14 mg/kg; H after injection of nembutal in dose of 5 mg/kg. Curves C, D, E, F, G, and H obtained during the same sweep of the beams. Time calibration for these—see H.

means of an ES-103 stimulator with radiofrequency output. The duration of each stimulus was 0.1 msec. Potentials evoked by stimulation of nerves at strengths 4 and 40 times greater than the threshold level for the appearance of a negative dorsal surface potential (4T and 40T) were studied.

EXPERIMENTAL RESULTS

The characteristics of the P waves evoked by stimulation of the sural nerve in both limbs of healthy rats and rats with tetanus are given in Table 1. In healthy rats no difference was found in the effects of stimulation of nerves on both sides, whereas in rats with local tetanus, a slight increase in the P wave produced by stimulation of the nerve on the "tetanus" side was observed. In rats with ascending tetanus, stimulation of the nerve on the "tetanus" side was accompanied by the appearance of a P wave whose amplitude and duration were several times greater than those of the P wave evoked by stimulation of the nerve on the opposite side (difference highly significant). To analyze the origin and composition of the augmented P wave in rats with a paroxysmal syndrome with ascending tetanus, a number of methods were used which, in the healthy animal, acted in different ways on the characteristics of the P wave and ED.

After injection of nembutal in a dose of 14 mg/kg into healthy rats and rats with local tetanus, the familiar action of barbiturates was observed—increased duration of the P wave because of the slowing of its decline and a decrease in amplitude of ED (Fig. 1A, B, C, and D). In rats with ascending tetanus, after injection of the same dose of nembutal, the duration of the P wave produced by stimulation of the nerve on the "tetanus" side was shortened, not lengthened (Fig. 1E and F). The paroxysmal discharge was shortened even more in the ventral root, as a result of which the coincidence in time between the ED and the P wave was disturbed (Fig. 1F). In contrast to the P wave of the healthy rat under nembutal, which fell steadily from its peak (Fig. 1B), the P wave in rats with ascending tetanus fell more steeply, and a flexure (marked by an arrow in Fig. 1F) could be seen in the phase of its decline, after which the character of the decline became similar to that in the healthy rats under nembutal. A further injection of the same dose of nembutal slowed the decline of the P wave after the flexure to an even greater degree, thus emphasizing it more clearly. After injection of a smaller dose of nembutal (5 mg/kg) into rats with ascending tetanus, shortening of the P wave was observed (Table 1; Fig. 1H), although no flexure could be seen in the phase of decline. These special features distinguishing the P wave evoked by stimulation of the nerve on the "tetanus" side in rats with a paroxysmal syndrome associated with ascending tetanus can be explained on the assumption that the P wave consists of two components: a component analogous to the P wave in the healthy animal and a paroxysmal component. It is the "paroxysmal" component which determines the duration of the composite P wave in the unanesthetized rat.

Under the influence of nembutal in a dose of 5 mg/kg, the "paroxysmal" component became shorter and the duration of the efferent discharge was less than that of the P wave (Fig. 1H). In moderate doses (14 mg/kg) nembutal shortened the discharge and the "paroxysmal" component still further, while lengthen-

TABLE 1. Characteristics of P Wave of Dorsal Surface Potential of Spinal Cord in Healthy Rats with Local and Ascending Tetanus

Animals investigated	Side of stimulation	Amplitude (in μV)						Duration (in msec)					
		4T			40T			4T			40T		
		n	$\bar{x}\pm S_{\bar{x}}$	P	n	$\bar{x}\pm S_{\bar{x}}$	P	n	$\bar{x}\pm S_{\bar{x}}$	P	n	$\bar{x}\pm S_{\bar{x}}$	P
Healthy rats	Right	12	400±21	>0.05	12	445±30	>0.05	12	82±5	>0.05	12	96±6	>0.05
	Left	12	397±39		12	440±68		12	79±5		12	104±6	
Rats with local tetanus	"Tetanus"	16	454±38	>0.05	16	519±40	>0.05	16	77±6	>0.05	16	105±8	>0.05
	Opposite	16	362±37		16	430±64		16	73±9		16	87±6	
Rats with ascending tetanus	"Tetanus"	24	1 242±73	<0.001	23	1 304±69	<0.001	24	525±34	<0.001	23	581±39	<0.001
	Opposite	17	413±48		17	546±51		17	64±4		17	82±8	
After injection of nembutal in dose of 14 mg/kg													
Healthy rats	Right	12	385±20	>0.05	12	445±21	>0,05	12	176±11	>0.05	12	193±13	>0,05
	Left	11	393±41		10	433±45		11	181±10		10	192±14	
Rats with local tetanus	"Tetanus"	15	434±44	>0,05	15	506±44	>0,05	15	169±16	>0,05	15	178±18	>0.05
	Opposite	15	365±36		15	415±39		15	157±10		15	163±4	
Rats with ascending tetanus	"Tetanus"	10	803±116	<0.01	10	959±100	<0.01	10	176±13	>0,05	10	171±17	>0,05
	Opposite	7	347±51		7	445±64		7	184±14		7	197±15	
After injection of nembutal in dose of 5 mg/kg													
Rats with ascending tetanus	"Tetanus"	5	1 510±290	<0,01	6	1 576±182	<0,01	5	110±18	>0,05	6	111±12	>0.05
	Opposite	5	342±67		5	475±82		5	79±13		5	80±13	

Legend: n denotes number of observations; $\bar{x}$ arithmetical mean; P probability of zero hypothesis. Level of significance taken as 0.05. Difference significant when $P < 0.05$.

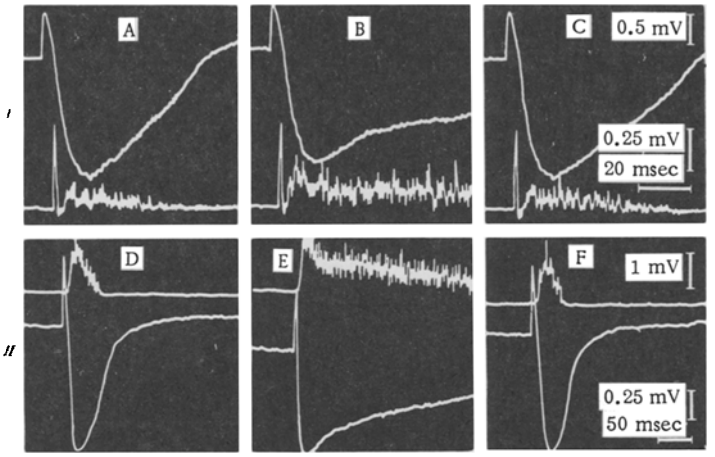

Fig. 2. Action of repetitive stimulation (I) and asphyxia (II) on DSPs and EDs in ventral roots of spinal cord in rats with ascending tetanus. I) Rat anesthetized with nembutal (5 mg/kg). In curves A, B, and C: DSPs shown above and EDs of root L_6 below; A) DSP and ED of root L_6 during stimulation of posterior tibial nerve on "tetanus" side at frequency of 1/10 sec and strength 40T; B) the same, frequency 1/2 sec; C) the same returning to stimulation at 1/10 sec; II) rat anesthetized with nembutal (14 mg/kg). In curves D, E, and F: EDs of root L_6 shown above, DSPs below; D) ED of root L_6 and DSP evoked by stimulation of sural nerve on "tetanus" side before beginning of asphyxia; E) the same 65 sec after switching off respirator; F) the same after recovery from asphyxia.

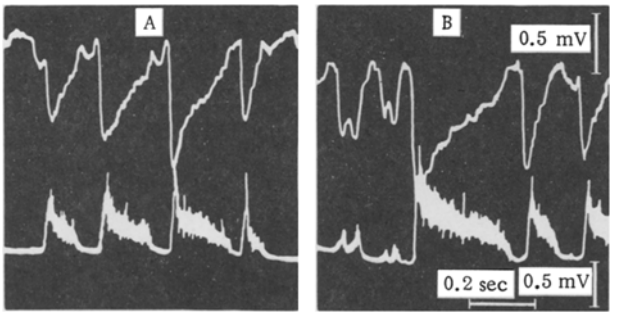

Fig. 3. Paroxysmal activity in a rat with ascending tetanus. In curves A and B: DSPs above, EDs of root L_5 on "tetanus" side below.

ing the usual component (Fig. 1B). The usual component was now longer in duration than the "paroxysmal" so that the duration of the composite P wave became equal to the duration of the P wave in the healthy rat and rats with local tetanus (Table 1; Fig. 1B, D, and F). Further proof of the existence of a "paroxysmal" component in the P wave of rats with ascending tetanus was obtained by the use of methods increasing the duration of the efferent discharge. When paired stimuli were applied the shortened P wave was lengthened to the same degree as the paroxysmal discharge, the facilitatory effect of the first stimulus on the duration of the response to the second possibly lasting for hundreds of milliseconds. Lengthening of the P wave also took place with the change from single to low-frequency repetitive stimulation (Fig. 2A, B, and C). In the course of rhythmic stimulation the duration of the P wave evoked by stimulation of the nerve on the tetanus side in rats with ascending tetanus sometimes increased, and then decreased parallel to the analogous changes in duration of the paroxysmal discharges in the ventral root. Lengthening of the P wave and ED in rats with ascending tetanus was clearly visible against the background of asphyxia (Fig. 2E). Just as when repetitive stimulation was used, the action of asphyxia was visible only during nembutal anesthesia.

In some rats with ascending tetanus, spontaneous prolonged surface potentials of the spinal cord and discharges in the ventral roots similar to those evoked by stimulation of the nerve on the "tetanus" side

were recorded. Because of the irregularity of their generation and their irregular shape, the connection between these potentials and the intensity and duration of the efferent discharges could be seen particularly clearly (Fig. 3A and B).

These experiments thus showed that in rats with ascending tetanus and a paroxysmal syndrome the P wave evoked by stimulation of a nerve on the "tetanus" side is heterogeneous and apparently consists of two components: a component analogous to the P wave in healthy animals, and a "paroxysmal" component. In this connection it is an interesting fact that in poisoning with strychnine, whose mechanism of action on the central nervous system is similar to that of tetanus toxin [6], strychnine-resistant and strychnine-sensitive components have been distinguished in the dorsal root potential [1, 7-9].

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