CHANGES IN TISSUE CONCENTRATION OF CATECHOLAMINES IN EXPERIMENTAL TETANUS

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The catecholamine concentration in the urine and in various tissues was studied in the course of experimental tetanus. A marked increase in the excretion of adrenalin and noradrenalin in the urine was discovered. This was accompanied by increased mobilization of catecholamines from the adrenals and their selective accumulation in the region of the hypothalamus. Administration of therapeutic doses of chlorpromazine considerably prolonged the survival of lethally poisoned animals, and restored the normal catecholamine concentration in their hypothalamus.

A previous investigation showed that osmoregulatory reflexes in response to hydration and dehydration of the body are disturbed in experimental tetanus [7]. This disturbance was shown to be associated with increased excitability of adrenergic neurons within the hypothalamo-hypophyseo-adrenal system (HHAS) [8].

The object of the investigation described below was to study changes in the excretion of adrenalin and noradrenalin in the urine in experimental tetanus and to determine whether any accumulation of catecholamines takes place under these circumstances in various parts of the HHAS.

EXPERIMENTAL METHOD

Experiments were carried out on dogs weighing 10-12 kg and cats weighing 3-3.5 kg. Tetanus toxin (1 MLD for mice 0.00005 mg of the dry toxin) was injected into dogs intravenously in a dose of 1 mg/kg, and into cats intramuscularly in a dose of 1.5 mg/kg. The first clinical manifestations of descending tetanus appeared in the dogs 48 h after injection of the toxin, and the tetanus became generalized after 96 h. In cats local tetanus developed on the side of injection of the toxin 48 h after its injection, and generalized tetanus developed 120 h after injection.

Urine was collected from the experimental dogs for a period of 6 h (from 9 a.m. to 3 p.m.) at the times of maximal excretion of catecholamines in the urine [4]. The tissue catecholamine concentration was determined at various times in the course of the poisoning in experiments on cats. The animals were decapitated and the tissues of the hypothalamus, medulla, spinal cord, suprasylvian gyrus of the cerebral cortex, cerebellum, liver, kidneys, heart, lungs, skeletal muscles, and sciatic nerve were rapidly removed for testing.

The catecholamine concentrations in the urine and tissues were determined by Men'shikov's method [6] on a type ÉFM fluorometer, with additional dc amplification with a factor of 8.

EXPERIMENTAL RESULTS

The experiments were divided into two series. In the first series the excretion of catecholamines in the urine was investigated. Noradrenalin excretion was increased (Table 1) in the prespastic stage of tetanus, when the adrenalin excretion still remained unchanged. The noradrenalin excretion returned to normal

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TABLE 1. Concentration of Catecholamines in 6-h Urine Sample from Dogs with Experimental Tetanus (in μg)

Time after injection of toxin	Adrenalin			Noradrenalin		
	number of determinations	M ± m	P	number of determinations	M ± m	P
Without administration of chlorpromazine						
Control	26	2.77 ±	0.33	19	4.52 ± 0.52	
24 h (absence of clinical manifestations)	14	4.82 ± 0.88	> 0.05	14	6.6 ± 0.75	< 0.05
48 h (rigidity of occipital muscles)	13	5.3 ± 0.72	< 0.01	13	5.3 ± 0.56	> 0.25
72 h (spasticity of all muscles)	14	5.3 ± 0.57	< 0.002	14	4.49 ± 0.65	> 0.5
96 h (generalized tetanus)	15	4.7 ± 0.22	< 0.05	14	4.6 ± 0.78	> 0.5
With administration of chlorpromazine						
48 h (absence of clinical manifestations)	8	10.2 ± 1.05	< 0.001	6	4.44 ± 0.68	> 0.5
72 h (rigidity of occipital muscles)	9	12.33 ± 1.06	< 0.001	7	13.33 ± 1.61	< 0.002
96 h (spasticity of all muscles)	7	10.41 ± 1.31	< 0.002	7	10.7 ± 1.51	< 0.01
120 h (generalized tetanus, convulsions)	6	12.16 ± 1.6	< 0.01	6	8.43 ± 1.43	< 0.01
144 h (generalized tetanus)	7	2.82 ± 0.68	> 0.5	7	3.15 ± 0.42	> 0.5

Note. P calculated relative to control.

after 48 h, but the adrenalin excretion in the urine was considerably increased. This relationship continued until the development of severe, generalized tetanus and death of the animals. Administration of chlorpromazine (3 mg/kg) to the dogs twice daily for 5 days [5] delayed the developement of poisoning: the incubation period was lengthened by approximately 1 day and as a rule the animals died 2-3 days later, with the same clinical manifestations as in the absence of chlorpromazine. In dogs receiving chlorpromazine the increased adrenalin excretion occurred a little sooner (48 h after injection of the toxin) and remained high for 3-4 days. Before death of the animals (usually 6-7 days after poisoning) the adrenalin excretion fell appreciably. The noradrenalin excretion increased 72 h after injection of the toxin and decreased only just before death of the animals.

The increase in catecholamine excretion in the urine under the influence of chlorpromazine thus had a depotentiating action on the development of experimental tetanus. This effect could be associated with the marked decrease in the catecholamine concentration in the tissues [2] and inhibition of metabolic activity [5], resulting in weakening of the antienzyme action of the toxin. To clarify this problem, the adrenalin and noradrenalin concentrations were determined in different organs and tissues of cats, in which the mechanisms of development of tetanus are the same as in dogs [1].

It will be clear from Table 2 that the greatest changes in catecholamine concentrations occurred in the hypothalamus: even in the early stage of poisoning, their concentration was higher than normal, reaching a maximum during the development of generalized tetanus. A very small increase in the adrenalin concentration was observed in the cerebral cortex. The catecholamine concentration in the adrenals fell sharply, especially in generalized tetanus. In the other organs (heart, liver, kidneys, lungs, spinal cord, medulla, sciatic nerve, and skeletal muscle) the adrenalin concentration was virtually unchanged ($P > 0.5$) in either the early or the late stages of poisoning. The noradrenalin concentration in these organs, however, was considerably reduced, especially in generalized tetanus ($P < 0.05$).

Administration of chlorpromazine to the cats retarded (just as in dogs) the development of experimental tetanus: local spasticity of the hind limb into which the toxin was injected developed about 24 h

TABLE 2. Concentration of Catecholamines in Tissues at Different Stages of Experimental Tetanus in Cats (in $\mu g/g$ tissue)

Time after injection of toxin	Adrenals						Kidneys		
	adrenalin			noradrenalin			adrenalin		
	number of determinations	$M \pm m$	P	number of determinations	$M \pm m$	P	number of determinations	$M \pm m$	P
Without administration of chlorpromazine									
Control	11	402,7±13,4		11	102,0±17,2		14	0,21±0,02	
48 h (local tetanus)	10	27,6±9	<0,001	10	86±5,5	>0,5	10	0,33±0,08	>0,1
72 h (local tetanus)	14	280,2±17,2	<0,001	11	92,5±5	>0,5	14	0,37±0,05	<0,05
96 h (spasticity of hind limbs)	12	132,0±7,2	<0,001	12	43±5	<0,01	12	0,371±0,05	<0,05
144 h (generalized tetanus)	17	42,4±3,7	<0,001	12	27±3,8	<0,002	12	0,28±0,04	>0,5
With administration of chlorpromazine									
72 h (local tetanus)	10	33,8±3,5	$\frac{P<0,001}{P_1<0,001}$	10	85,5±4,9	$\frac{P>0,5}{P_1>0,5}$	11	0,23±0,03	$\frac{P>0,5}{P_1<0,05}$
144 h (generalized tetanus)	18	84,6±11	$\frac{P<0,001}{P_1<0,01}$	13	56,0±10,7	$\frac{P<0,05}{P_1<0,05}$	18	0,19±0,03	$\frac{P>0,5}{P_1<0,05}$

(Continuation)

Time after injection of toxin	Kidneys			Hypothalamus					
	noradrenalin			adrenalin			noradrenalin		
	number of determinations	$M \pm m$	P	number of determinations	$M \pm m$	P	number of determinations	$M \pm m$	P
Without administration of chlorpromazine									
Control	12	0,9±0,11		18	0,63±0,05		15	0,45±0,03	
48 h (local tetanus)	10	0,61±0,08	<0,05	10	0,72±0,08	>0,5	10	0,69±0,09	>0,05
72 h (local tetanus)	10	0,62±0,05	<0,05	14	0,87±0,13	<0,05	10	0,79±0,04	<0,001
96 h (spasticity of hind limbs)	8	0,32±0,05	<0,01	12	1,36±0,12	<0,001	7	0,72±0,11	<0,001
144 h (generalized tetanus)	10	0,25±0,06	<0,01	17	1,25±0,05	<0,001	9	0,92±0,13	<0,001
With administration of chlorpromazine									
72 h (local tetanus)	8	0,49±0,4	$\frac{P<0,02}{P_1<0,05}$	11	0,9±0,07	$\frac{P<0,05}{P_1>0,5}$	6	0,41±0,06	$\frac{P>0,5}{P_1<0,01}$
144 h (generalized tetanus)	10	0,15±0,02	$\frac{P<0,001}{P_1>0,5}$	18	0,77±0,1	$\frac{P>0,25}{P_1<0,01}$	10	0,26±0,04	$\frac{P<0,02}{P_1<0,002}$

Note. P reflects difference from control, P_1 difference from corresponding stage of tetanus without administration of chlorpromazine.

later than in the untreated animals, and the duration of their survival until death from generalized tetanus was 2-3 days longer than in the control. Because of the later development of local tetanus, catecholamines in the tissues were determined on the third day, and in the case of generalized tetanus, on fifth to sixth day after injection of the toxin. In this way the results could be compared on the basis of similarity of the clinical picture in the animals receiving and not receiving chlorpromazine. After administration of therapeutic doses of chlorpromazine, the increased duration of survival of the animals was accompanied by only a transient increase in the adrenalin concentration in the hypothalamus at the stage of local tetanus, whereas the noradrenalin concentration was lowered. As the effect of the toxin became generalized, the concentration of catecholamines in this part of the brain fell. Administration of chlorpromazine did not prevent mobilization of catecholamines from the adrenals. In the other organs (spinal cord, cerebellum, liver, heart, kidneys, lungs, medulla) the adrenalin concentration was unchanged compared with its level in poisoned animals not receiving chlorpromazine, whereas the noradrenalin concentration fell significantly ($P < 0.02$).

It can be concluded from these results that the varied pattern of disturbance of catecholamine metabolism in the tissues in experimental tetanus leads to potentiation of the pathogenic action of the tetanus toxin. On the other hand, the decrease in the catecholamine concentration in the tissues, notably in the hypothalamus, under the influence of chlorpromazine delays the development of experimental tetanus. The disturbances of catecholamine metabolism discovered in experimental tetanus are evidently nonspecific in character. Experiments with strychnine poisoning (1 mg/kg daily for 2 days) showed that during the development of muscular spasticity and convulsions the concentration of catecholamines in the hypothalamus also was increased, while the noradrenalin concentration in several organs (cerebellum, heart, liver, kidneys, medulla, adrenals) was higher ($P < 0.05$) than in tetanus.

LITERATURE CITED

1. G. N. Kryzhanovskii, Tetanus [in Russian], Moscow (1966).
2. É. I. Lipovetskaya, Vopr. Pitaniya, No. 2, 81 (1962).
3. K. M. Loban, The Treatment of Tetanus [in Russian], Moscow (1965).
4. É. Sh. Matlina, Z. M. Kiseleva, and I. E. Sofieva, in: Methods of Investigation of Some Hormones and Mediators [in Russian], Moscow (1965), p. 25.
5. G. I. Meerov, Vopr. Med. Khimii, No. 6, 611 (1966).
6. V. V. Men'shikov, Lab. Delo, No. 4, 18 (1961).
7. V. V. Mikhailov and N. P. Chesnokova, Pat. Fiziol., No. 1, 48 (1968).
8. V. V. Mikhailov and N. P. Chesnokova, Pat. Fiziol., No. 3, 42 (1968).