OXIDATIVE PHOSPHORYLATION IN THE SKELETAL MUSCLES OF RABBITS DURING DINITROPHENOL HYPERTHERMIA

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After intramuscular injection of 2,4-dinitrophenol (DNP) into rabbits in doses of 10, 15, 25, and 30 mg/kg the degree of elevation of the body temperature, the increase in the oxygen consumption, and the degree of uncoupling of oxidative phosphorylation in skeletal muscle homogenates were found to increase with the dose. In the course of dinitrophenol hyperthermia (following injection of 25 mg/kg DNP) the changes in body temperature and oxygen consumption of the animals followed a parallel course with the changes in the level of oxidative phosphorylation in the skeletal muscles.

KEY WORDS: dinitrophenol hyperthermia; oxidative phosphorylation; rabbit skeletal muscles; oxygen consumption.

The cause of the hyperthermia in animals poisoned with 2,4-dinitrophenol (DNP) is a sharp toxic increase in heat production in the tissues, linked nowadays with a disturbance of oxidative phosphorylation in the tissues [4, 5, 8, 9]. However, the data on the state of these processes in tissue preparations isolated from animals receiving DNP are contradictory [1, 3, 6, 8, 11].

These contradictions can evidently be explained by differences in the experimental conditions: differences in the experimental animals used, the times after injection of the DNP, the methods of obtaining mitochondria, the final concentrations of the tissue products (and, consequently, of DNP) in the incubation mixture, and so on. The uncoupling action of DNP when added to isolated mitochondria can be abolished if the poison is removed during subsequent washing of the mitochondria [7, 10]. During isolation of mitochondria from the liver and skeletal muscles of animals poisoned with DNP, most of the DNP therefore passes into the soluble phase and the amount of the poison absorbed on the mitochondria is insufficient to inhibit oxidative phosphorylation [6]. In experiments on liver slices and skeletal muscle homogenates from animals receiving DNP, the P/O ratio was in fact lowered [3].

This paper describes the results of a study of the level of oxidative phosphorylation in skeletal muscle homogenates from rabbits and compares them with changes in the body temperature and the oxygen consumption of the animals at various times after receiving DNP.

EXPERIMENTAL METHOD

Rabbits weighing 2.2-3.2 kg were used. The body temperature and oxygen consumption were measured by Veselkin's method [2] before and after injection of DNP into the animals. The DNP was injected intramuscularly as a 1% solution in doses of 10, 15, 25, and 30 mg/kg. At the end of the experiment the animals were decapitated, the gastrocnemius muscle was quickly excised, membranes and fat were removed from it, and the muscle was minced with scissors for 4 min. Oxidative phosphorylation was studied during incubation of the tissue preparations in a Warburg apparatus at 37°C in an atmosphere of air. The incubation mixture contained the following components (in moles): potassium-phosphate buffer, 40, KCl 138, $MgCl_2$ 10, glucose 150, Na_2-EDTA 3, Tris-HCl (pH 7.4) 50, succinic acid 60, Na_2-ATP 6.2, hexokinase 300 μg.

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TABLE 1. Body Temperature (T) and Oxygen Consumption (O_2) of Rabbits and P/O and Q_{O_2} Coefficients for Skeletal Muscle Homogenates 2 h after Injection of Various Doses of DNP into Animals (M ± m)

Parameter studied	Control	After injection of DNP in doses given below (in mg/kg)			
		10	15	25	30
T	38,9±0,02 (59)	39,2±0,07 (12)	39,1±0,05 (10)	40,0±0,14 (16)	40,6±0,24 (7)
P		<0,001	<0,001	<0,001	<0,001
O_2	10,1±0,10 (37)	11,6±0,42 (5)	11,0±0,20 (10)	16,7±0,60 (16)	19,4±0,82 (7)
P		<0,001	<0,001	<0,001	<0,001
P/O	1,83±0,03 (29)	1,63±0,14 (7)	1,32±0,13 (10)	1,02±0,20 (7)	0,74±0,14 (7)
P		>0,5	<0,01	<0,002	<0,002
Q_{O_2}	485±12 (29)	551±27 (7)	571±14 (10)	646±63 (7)	543±44 (7)
P		<0,02	<0,002	<0,02	<0,5

Note. Here and in Table 2, P represents significance of difference compared with controls; number of experiments given in parentheses.

The quantity of muscle homogenate taken was 900 mg. Tissue respiration was measured manometrically and expressed in microatoms oxygen utilized during incubation and also in microliters oxygen consumed during incubation for 1 h by 1 mg tissue (Q_{O_2}). Phosphorylation was judged by the difference between the content of mineral phosphate in the incubated and unincubated samples; phosphorus was determined by the method of Fiske and Subbarow [12] and expressed in microatoms. The P/O ratio was calculated from the results obtained.

EXPERIMENTAL RESULTS AND DISCUSSION

Injection of DNP in the various doses into the animals caused a rise in the body temperature and in the oxygen consumption (Table 1). After doses of DNP of 10-15 mg/kg the body temperature rose by only 0.2-0.3°C and the oxygen consumption rose by 9-15%. With doses of 25 and 30 mg/kg the body temperature rose by 1.1 and 1.7°, respectively, while the oxygen consumption increased by 65 and 92% above the initial level.

An increase in the oxygen utilization by the skeletal muscles of the rabbits poisoned with DNP also was observed; with an increase in the dose of the poison injected from 10 to 25 mg/kg the stimulation of respiration was increased by 14 and 33%, respectively (Table 1). Conversely, the level of oxidative phosphorylation in muscle homogenates from the poisoned animals decreased; the P/O ratio after administration of 30 mg/kg DNP was 60% lower, but after a dose of 10 mg/kg only 11% lower than the control.

Since DNP in doses of 25 and 30 mg/kg caused practically the same degree of uncoupling of oxidative phosphorylation in the rabbit skeletal muscle homogenates, and in view of data [13] showing that rabbits often die after receiving DNP in a dose of 30 mg/kg, the main series of experiments was carried out with a dose of 25 mg/kg.

As Table 2 shows, 30 min after the injection of DNP the animals' body temperature was raised by 1°C, at the end of 1 h on the average it was 1.2°C above its initial value, and it remained at about the same level until the end of the second hour, after which it fell gradually to reach its initial level after 5 h. The oxygen consumption of the animals also rose (by 53%) 30 min after the injection of DNP, it reached a maximum (+79%) after 1 h, it was still 60% above the initial level after 2 h, and then it fell gradually and returned to its initial level 5 h after injection of the poison.

Respiration in the muscle homogenate from the animals poisoned with DNP was 27-44% higher than in the control animals throughout the 5 h of observation. The greatest decrease (60%) in the P/O ratio in the skeletal muscles was observed 1 h after the injection of DNP, i.e., at the time of the highest rise of the body temperature. After 2 h, when the body temperature still remained high and the oxygen consumption of

TABLE 2. Body Temperature (T) and Oxygen Consumption (O_2) of Rabbits and P/O and Q_{O_2} Coefficients for Skeletal Muscle Minces at Various Times after Injection of DNP in a Dose of 25 mg/kg into Animals (M ± m)

Parameter studied	Control	After injection of DNP in doses given below (in mg/kg)					
		0,5	1	2	3	4	5
T	38,8±0,03 (49)	39,8±0,06 (8)	40,0±0,08 (23)	40,0±0,14 (16)	39,6±0,01 (9)	39,1±0,09 (9)	38,9±0,08 (9)
P		<0,001	<0,001	<0,001	<0,001	<0,2	<0,5
O_2	10,4±0,12 (37)	15,9±0,6 (7)	18,6±0,56 (23)	16,7±0,60 (16)	13,1±0,55 (9)	11,8±0,33 (9)	10,5±0,29 (9)
P		<0,001	<0,001	<0,001	<0,001	<0,001	>0,5
P/O	1,77±0,04 (19)	1,01±0,11 (8)	0,72±0,08 (7)	1,02±0,20 (7)	—	—	1,36±0,12 (9)
P		<0,001	<0,001	<0,01			<0,002
Q_{O_2}	448±15 (19)	599±9 (8)	572±34 (7)	646±63 (7)	—	—	612±19 (9)
P		<0,001	<0,01	<0,01			<0,001

the rabbits was only very slightly reduced, the P/O ratio rose slightly but still remained 40% below its control value. The level of oxidative phosphorylation in the skeletal muscles 5 h after the injection of DNP remained 25% below the control level.

Comparison of the dynamics of the changes in the parameters studied during poisoning of the animals with DNP showed that they are closely interconnected. The uncoupling of oxidative phosphorylation and the accumulation of dephosphorylation products of ATP were evidently the causes of the stimulation of respiration in the tissue preparations and in the body as a whole, with a consequent increase in heat formation during dinitrophenol poisoning.

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