ALLERGOLOGY

Possible Mechanisms of Allergic Reactions of the Skeletal Muscle

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The effects of denervation and sensitization alone and in combination on the isometric contractions of guinea pig diaphragmal muscle are studied *in vitro*. Denervated diaphragmal muscle contracts in response to histamine, the reaction being abolished by the histamine receptor blocker dimedrol. After sensitization, these muscles respond to histamine and specific antigen, contraction being prevented by incubation with dimedrol and the mast cell blocker Intal. The possible mechanisms of reactions in the muscles are discussed.

Key Words: *skeletal muscle; sensitization; denervation; anaphylactic contraction; mast cell*

The morphofunctional state of skeletal muscles in allergic disease has been poorly investigated [4,10]. Meanwhile, allergic reactions of skeletal muscles may contribute to the pathogenesis of some disease, for example, bronchial asthma [12]. It was suggested that diaphragmal dysfunction is linked with respiratory disorders [3].

Anaphylactic contraction (AC) is one of the manifestations of allergic reaction in the skeletal muscle. The mechanisms of AC are not fully understood [10]. This type of contraction in the skeletal muscle was originally described in 1944 [1]. Contractions of denervated muscles of the tongue in response to resolving dose of antigen were observed in dogs sensitized with horse serum. Similar results were obtained in experiments with denervated diaphragmal muscle *in vitro* [7]. Anaphylactic contractions of denervated skeletal muscles are induced by histamine, serotonin, and other biologically active compounds [4]. It should be noted that AC occur only in denervated muscles and are induced by histamine, which is consistent with increased sensitivity of denervated muscles to this compound [11]. The mechanisms responsible for the histamine-induced contraction of denervated muscle are unclear.

In humans and most animal species, mast cells are the major source of histamine in anaphylactic reactions [5]. Their role in allergic reactions involving smooth muscles was studied in sufficient detail [2], while their contribution to the development of AC of the skeletal muscles was not assessed. Assuming that histamine released from mast cells triggers anaphylactic reactions in the skeletal muscle, one should expect that these reactions can be abrogated by histamine blockers and preparations preventing mast cell degranulation.

In order to evaluate the role of histamine and mast cells on AC of the skeletal muscle, we examined the effects of Intal (cromolyn sodium) and the histamine receptor blocker dimedrol on the guinea pig diaphragmal muscle. Better understanding of the mechanisms underlying the effects of these drugs on the skeletal muscle may be useful for an adequate choice of anti-allergic therapy.

MATERIALS AND METHODS

Experiments were performed on male guinea pigs weighing 250-350 g. They were sensitized by two

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TABLE 1. Concentrations, Doses, and Incubation Times with the Studied Compounds

Compound	Concentration	Incubation time
Carbacholine	2×10^{-4} M	-
Histamine	2×10^{-4} M	-
Ovalbumin	500 mg/ml	-
Dimedrol	1×10^{-6} M	3 min
Intal (cromolyn sodium)	2×10^{-4} M	20 min

subcutaneous injections of 10 μg ovalbumin and 1 mg dry aluminum hydroxide gel in 1 ml normal saline per animal at a 14-day interval. The level of sensitization was controlled by passive cutaneous anaphylaxis [8] and thin-layer immune analysis [9]; on day 21, the antibody titers were 1/256-1/1024 and 1/25-1/512, respectively.

For denervation of the diaphragmal muscle, the diaphragmal nerve was dissected, ligated, and a 0.5-cm long segment was cut out. In experiments with denervation and sensitization, the animals were injected with ovalbumin on day 1 after surgery. The contractile response of the left diaphragmal muscle (*m. phrenicus*, a 5-mm wide stripe) was studied *in vitro* in the isometric regime as described previously [6]. The following parameters were measured: latent period, time of the maximum tension development, rate of contraction, force of contraction, half-time of relaxation, and duration of the plateau. Contractions were induced by the addition of carbacholine, histamine, or specific antigen (ovalbumin) to the incubation medium. The following control series of experiments were performed:

1. Incubation of the diaphragmal muscle preparation from intact and sensitized animals with dimedrol.

2. Incubation of the diaphragmal muscle preparation from intact, sensitized, and denervated animals with Intal.

When the muscle did not contract in response to any of the inducers, their effects were studied after a 20-min washout with the incubation medium. The concentrations of the studied compounds and incubation times are given in Table 1.

Muscles were studied three weeks after the beginning of experiment. Results were analyzed using Statgrafics software.

TABLE 2. Parameters of Isometric Contraction of Guinea Pig Diaphragmal Muscle Under Various Experimental Conditions ($X\pm Sx$)

Experimental conditions	Contraction parameters					
	latency, sec	maximum tension time, sec	contraction force, mg	contraction rate, mg/sec	relaxation half-time, sec	plateau duration, sec
Carbacholine, 2×10^{-4} M						
Control (n=16)	4.03±0.40	13.60±0.61	147.33±15.4	10.82±0.95	15.55±0.73	5.33±0.83
Denervation (n=8)	3.00±0.61	11.50±1.22	182.50±46.42	18.69±6.92	11.88±0.83	3.38±0.65
Sensitization (n=11)	1.82±0.15	21.00±1.20	426.0±37.90	20.23±1.46	17.73±1.01	5.36±0.73
Denervation and sensitization (n=8)	1.13±0.35	8.13±1.59	230.6±63.53	29.92±8.46	9.50±1.88	2.88±0.35
Intal (n=5)	4.60±0.6	19.20±4.09	186.80±32.31	12.09±3.39	25.40±4.26	12.20±2.33
Denervation and sensitization+Intal (n=13)	1.69±0.31	10.15±1.51	229.38±67.80	19.31±4.60	21.62±6.58	15.62±4.30
Dimedrol (n=8)	2.37±0.32	19.38±2.06	242.94±36.32	13.11±2.28	24.00±4.14	9.63±1.46
Denervation+dimedrol (n=4)	2.00±0.41	12.50±2.22	232.50±25.52	19.33±1.44	17.50±1.32	4.25±0.63
Denervation and sensitization+dimedrol (n=4)	1.25±0.25	3.75±0.48	135.0±10.41	37.38±4.48	13.50±1.19	1.75±0.48
Histamine, 2×10^{-4} M						
Denervation and sensitization (n=8)	10.50±1.66	19.33±3.48	154.13±32.41	8.84±3.56	40.00±13.23	15.00±5.00
Denervation and sensitization (n=8)	6.00±0.71	21.75±2.69	243.75±27.24	11.53±1.30	25.50±5.07	15.50±3.69
Denervation and sensitization+Intal (n=13)	31.13±7.41	13.50±4.08	21.23±3.29	2.83±1.20	6.50±1.30	11.69±2.90
Antigen (ovalbumin, 500 μg/ml)						
Denervation and sensitization (n=8)	36.50±7.76	17.37±2.81	48.12±11.01	2.83±0.50	31.62±12.30	9.25±1.70

RESULTS

The diaphragmal muscle of intact guinea pigs contracted only after the addition of carbacholine to the incubation medium and did not respond to histamine and the specific antigen (ovalbumin). Denervated diaphragmal muscle responded to both carbacholine and histamine (Table 2).

Sensitization of intact animals induced no sensitivity of the diaphragmal muscle to histamine or ovalbumin; it contracted only in response to carbacholine (Table 2).

Anaphylactic contractions were induced by the resolving dose of ovalbumin in denervated diaphragmal muscle of sensitized animals. This preparation also reacted to histamine and carbacholine (Table 2).

Preincubation of denervated muscle with dimedrol prevented its contractions in response to histamine but not to carbacholine. Preincubation of denervated muscle from sensitized animals with dimedrol abolished contractions in response to histamine and the resolving dose of ovalbumin.

Experiments with Intal confirmed the participation of mast cells in AC of denervated diaphragmal muscle from sensitized guinea pigs. Intal prevented AC in response to ovalbumin and did not change its reaction to histamine and carbacholine.

Control experiments showed that neither dimedrol nor Intal prevents contractions of intact diaphragmal muscle in response to the studied agonists (Table 2). Intal had no effect on the ability of the diaphragmal muscle to contract in response to carbacholine and histamine.

In this report we do not discuss the parameters of contraction in detail. Meanwhile, these parameters illustrate the possibility of contraction in response to a given agonist (Table 2). At the same time, they are of special interest for the understanding of the precesses occurring in the skeletal muscle after denervation and sensitization.

Allergic processes involving mast cells are classified as anaphylactic reactions. Consequently, contraction of denervated diaphragmal muscle of sensitized guinea pigs can be regarded as anaphylactic contraction.

Our results indicate that sensitization changes considerably the contractile parameters of the skeletal muscles (Table 2), which agrees with our previous observation that sensitization affects the myosin spectrum [3,6].

Direct action of histamine on denervated muscle fibers was confirmed by experiments with dimedrol, which is consistent with increased sensitivity of denervated muscle to histamine [11].

The participation of mast cells in allergic contractions of the skeletal muscle was confirmed by experiments with Intal, a preparation that prevents degranulation of mast cells. Preincubation with Intal prevented AC of denervated diaphragmal muscle of sensitized animals in response to specific antigen and did not abolish the effect of histamine. This finding indicates that anaphylactic contractions of the skeletal muscle are induced by histamine released from mast cells.

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