Inhibition of Cardiac Muscle Contractions by Ultraviolet Light Irradiation

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ABSTRACT. Contraction of bullfrog atrial muscle, frog ventricular muscle, and rat papillary muscle were inhibited by ultraviolet (UV) light irradiation of weak intensity (75 mW/cm^2). The muscle preparation was superfused with the standard Ringer or Tyrode solution and repetitively stimulated at a rate of 1/2 sec or 1/5 sec. Shortly after irradiation on muscle with UV light, the twitch tension started to decrease to the steady level of 90% of the control tension within a few min. The tension recovered completely from inhibition if the UV irradiation was interrupted. This inhibition was enhanced when the superfusion of muscle preparation was stopped. Although there were no qualitative differences in twitch inhibitions among three kinds of muscle preparations, the rat papillary muscle was a little more sensitive to UV light than other muscles. The action potential recorded with a single sucrose gap method was prolonged in its tail without changes in plateau level and duration during UV irradiation. It is suggested that a certain injurious substance(s) which inhibits contraction is released by UV irradiation, rather than that UV damages the cellular structure. The substance has not yet been identified, but it is probably related to free radicals.

Key words: cardiac muscle contraction — ultraviolet light irradiation

It is well known that ultraviolet (UV) light has injurious effects on many kinds of cell functions. In frog skeletal muscle, Azuma[1] demonstrated that UV irradiation decreased a twitch tension and finally caused an irreversible contracture. Later Endo,[2] using both intact and skinned fibers of frog skeletal muscle, explained that damages of the sarcoplasmic reticulum (SR) and subsequent leakage of Ca^{2+} into myoplasm were the mechanisms of high power irradiation. In contrast with the excitation-contraction coupling of the skeletal muscle in which SR plays an exclusive role, Ca^{2+} entry into the cell during an action potential and the Na^{+}-Ca^{2+} exchange mechanism as well as the SR function are involved in the cardiac muscle. These mechanisms are possibly affected by UV irradiation. Moreover, recent studies have elucidated that the reperfusion injury after coronary artery occlusion is triggered by a chain reactions of free radicals produced in ischemic tissues.[3,4] As UV irradiation initiates the reaction easily, experiments on UV will provide some explanations of the reperfusion injury. The present work was carried out to examine the effects of UV irradiation on the contraction of isolated frog and rat cardiac muscles. Some differences among the experimental animals were expected because a frog ventricular muscle has poor SR whereas a rat papillary muscle

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has well developed one.[5] Some of the results were presented previously.[6]

TABLE 1. Effect of UV irradiation on twitch tension in different kinds of muscles.

Muscles	Stimulation frequency	Superfusion of muscle	Tension, % of the control mean ± SD	N
bullfrog atrium	1/5 sec	yes	87 ± 9.9	16
		no	64 ± 13.5	9
frog ventricle	1/5 sec	yes	90 ± 6.9	5
		no	76 ± 6.8	6
rat papillary	1/2 sec	yes	88 ± 10.0	6
		no	68 ± 14.3	5

METHODS

A thin muscle bundle of 0.3 to 0.4 mm in diameter and 3 to 4 mm in length was prepared from an inner wall of a bullfrog atrium and from a strip of a frog ventricle. Papillary muscle was isolated from the right ventricle of five- to eight-week-old rats after anesthesia with intraperitonial injection of sodium pentobarbital (30 mg/kg). The muscle preparation was placed horizontally in plastic chamber (5×5×50 mm), and was continuously perfused with oxygenated Ringer or Tyrode solution, and it was massively stimulated through Ag-AgCl plate electrodes with a current pulse of 3 msec duration and 2× supramaximal strength. One end of the muscle preparation was fixed and another end was tied to a capacitance tension transducer (Model 400, Cambridge Technology Inc., Cambridge, MA). The source of the UV light was a low power Hg-lamp of 75 mW/cm^2 (ELC-400, Electron-lite Co., Dunbury, CT). It emitted the maximum intensity at a wave length of 360 nm. The illuminated area was a circle with a diameter of 8.0 mm, which covered whole area of a muscle preparation.

The membrane potentials of a bullfrog muscle were recorded by the single sucrose gap method. The muscle preparation was divided into three portions; one end portion was continuously perfused with the standard Ringer solution, another end portion was perfused with 95 mM K_2SO_4 solution containing 8.0 mM $CaCl_2$, and the middle portion was perfused with isotonic sucrose (220 mM) solution. The muscle was stimulated across both end portions through Ag-AgCl wire electrodes and the resulting action potential was recorded with the same electrodes.

The Ringer solution was composed of (mM) NaCl 117, KCl 2.0, $CaCl_2$ 1.8, $NaHCO_3$ 4 and HEPES buffer 6 (pH=7.2), and the Tyrode solution contained NaCl 140, KCl 5.0, $CaCl_2$ 2.5, $MgCl_2$ 0.2, NaH_2PO_4 0.32 and HEPES buffer 6 (pH=7.2). The experiment was carried out at room temperatures of 20 to 24 ℃ for frog muscle, and at 33 ℃ for rat muscle.

RESULTS

1. Bullfrog and frog muscles

The experiments were undertaken soon after isolation of the muscle from a heart. The muscle was held at a slightly stretched condition so that the maximum tension might be developed, and it was stimulated repetitively at a rate of 1/5 sec. As was well known as a staircase phenomenon, the tension was small at first, increased progressively and attained the steady level in a few min. UV irradiation was started after the staircase phenomenon was completed. Figure 1, A shows a series of twitches of a bullfrog atrial muscle stimulated at 1/5 sec. Upon irradiation, the twitch tension started to decrease within a few tens of seconds, but recovered completely after the interruption of irradiation. The twitch inhibition due to irradiation could be repeated several times. Usually, fresh muscles shortly after isolation were less affected by UV irradiation than old muscles several hours after isolation. The twitch inhibition was enhanced if the perfusion through the muscle chamber with Ringer solution was suspended (Fig 1-B). Three out of 16 preparations of the

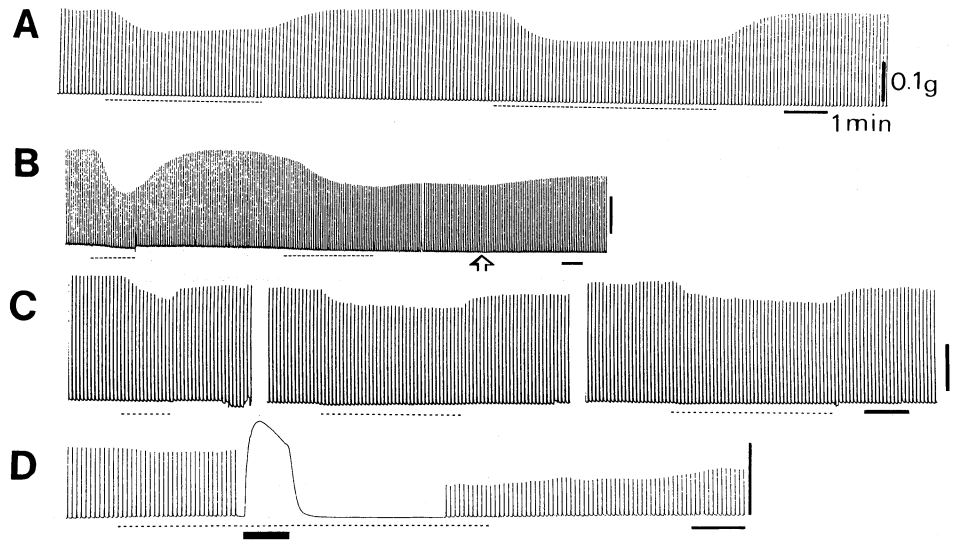

Fig 1. Changes in isometric tensions of bullfrog and frog cardiac muscles during UV irradiation for the period indicated by the broken lines.
A: Bullfrog atrial muscle was exposed to UV irradiation two times. No difference between the effects of the first and second irradiation. The muscle was continuously superfused with Ringer solution. 21℃. B: Bullfrog atrial muscle was UV irradiated when it was not superfused. Twitch tension recovered partly after a start of superfusion (indicated by the upward arrow). 21℃. C: Frog ventricular muscle was exposed to UV irradiation three times, while it was not perfused with Ringer solution. 24℃. About 10 min were interposed between each panel. D: Perfusion solution was exchanged from the standard Ringer solution to caffeine-high Ca (20 mM caffeine+10.8 mM $CaCl_2$) Ringer solution during the period indicated by a thick line, to see whether caffeine contracture takes place under UV irradiation. Bullfrog atrial muscle. 22℃. Calibration bars are 0.1g and 1 min in all records.

bullfrog atrial muscles showed no changes in tension during UV irradiation. The amount of the inhibition of tension was 0-20% of the twitch tension when the muscle preparation was superfused with Tyrode solution, whereas it was up to 40% when the superfusion was suspended. Twitches of the frog ventricular muscles were also inhibited in a similar manner to the atrial muscles (Fig 1-C). The results are summarized in TABLE 1.

Caffeine is known not only to augment twitch tension but also to cause contracture of cardiac muscle if it is applied together with $CaCl_2$.[7] When caffeine and $CaCl_2$ were applied during UV irradiation on muscle, it developed contracture in the similar amplitude and time course to those of the intact one.

The action potential during UV irradiation was recorded with the single sucrose gap method. The tail of the action potential was considerably prolonged three minutes after turning on UV irradiation. Changes in peak amplitude and in plateau duration were not observed (Fig 2).

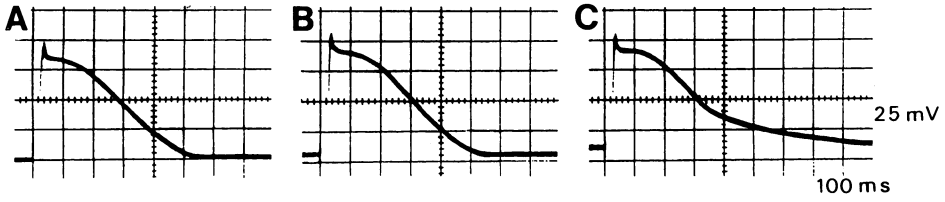

Fig 2. Action potentials of the bullfrog atrial muscle recorded by the sucrose-gap method. A: control. B: 1 min after, and C: 3 min after the start of UV irradiation.

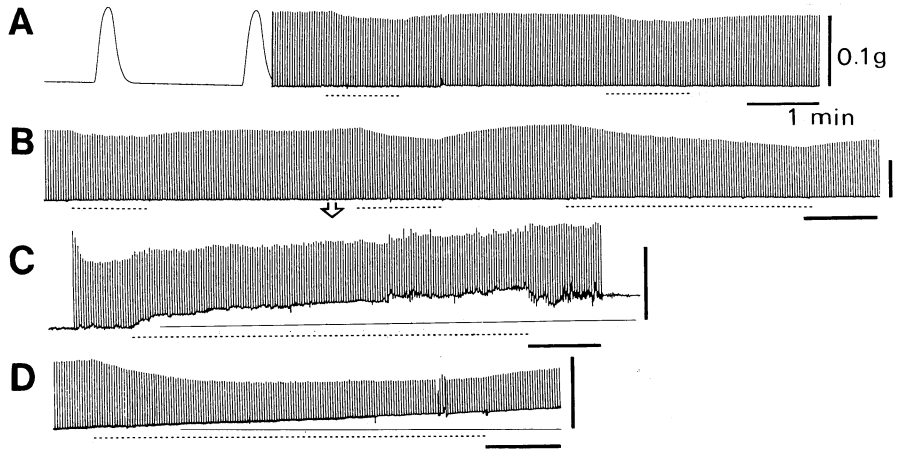

Fig 3. Changes in isometric tensions of rat papillary muscle during UV irradiation for the period indicated by the broken lines. A: The muscle was continuously superfused with Tyrode solution. Two series of exposures to UV light. B: At first, superfusion of muscle was continued and then it was suspended at the time shown by the downward arrow. C and D: Twitch inhibition and a rise of the diastolic tension induced by UV irradiation when muscle was not perfused. This horizontal lines indicate the initial tension level. Calibration bars are 0.1 g and 1 min except for the left record in A (1 sec). 33°C in all records.

2. Rat papillary muscle

The twitch tension of the rat papillary muscle was also inhibited by UV irradiation with good reversibility (Fig 3-A). It may be worth noting here again that the inhibitory effect was enhanced if the perfusion through the muscle chamber with Tyrode solution was suspended, as shown in Fig 3-B. If the UV irradiation continued beyond 5 min, the twitch tension was further decreased and the recovery was incomplete (Fig 3-B, D). In some preparations, the spontaneous activities, fluctuations of peak tension (Fig 3-C), and a rise of the diastolic tension level (Fig 3-D) took place in addition to the twitch inhibition.

DISCUSSION

The present report demonstrated that UV irradiation inhibited the twitch tension in frog and rat cardiac muscles. The twitch inhibition appeared rapidly, was enhanced by suspension of superfusion of muscle, but disappeared completely after interruption of irradiation. The likely explanation is that UV light produces a substance(s) which impairs the contraction processes. Several causes of the effect of UV irradiation can be proposed.

First, the substance may affect the chemical reactions in the plasma membrane. It has been reported that oxygen-derived free radicals reduce the number of calcium channels,[8] or shorten the action potential duration.[9] The present study showed prolongation of the action potential. Probably, this is because of a block of Ca^{2+}-activated K_1 current. However, any changes in duration or level of plateau of the action potential suggesting a decrease in Ca^{2+}-inflow were not observed.

Chen and Gills[10] reported the relaxation of vascular smooth muscle due to UV irradiation (366 nm). They suggested that the UV light released a certain factor or yielded H_2O_2, either of which activated guanylate cyclase, leading to an increase of intracellular cyclic GMP. Since a rise of the cGMP level decreases contractility of the rat heart[11] and frog ventricle,[12] it is possible that cGMP is the highly possible candidate responsible for twitch inhibition. This is the most probable explanation for the effect of UV irradiation. Studies on the action of the UV irradiation on oxygen free radical scavengers are required.

The third explanation is that the SR membrane becomes liable to leak out stored Ca into myoplasm after UV irradiation. A rise of the diastolic tension level shown in Fig 3-C probably reflects an increase of myoplasmic Ca^{2+} during diastolic period. At first, the rat muscle which has well developed SR was expected to be more sensitive to UV irradiation than the frog ventricular muscle provided with only poor SR. However, the marked difference of the effects of UV irradiation was not observed between two kinds of muscle preparation. Moreover, caffeine, which facilitates Ca^{2+}-induced Ca^{2+} release mechanism, produced contracture even though the muscle was previously irradiated by the UV light. Therefore, the contribution of SR to the twitch inhibition seems not to be a principal cause of the twitch inhibition in this study. This explanation may be different from Endo's conclusion,[2] who irradiated a single muscle fiber with focused UV light for microscopic observation. The UV light intensity in this study would be too weak to damage such intracellular structure as SR. Studies on UV light with different

intensities should be required. Neither is it likely that decomposition of riboflavin is involved in twitch inhibition, because the effect of UV irradiation appeared fairly rapidly.

The frog and bullfrog muscles exhibit seasonal variations in its sensitivity to UV irradiation. Autumn frogs were more sensitive to UV than winter or spring ones.

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