X-ray diffraction study on mammalian visceral smooth muscles in resting and activated states

M. WATANABE¹, S. TAKEMORI¹ and N. YAGI²

¹Department of Physiology, The Jikei University School of Medicine, Nishishinbashi, Minato-ku, Tokyo 105, Japan
²Department of Pharmacology, Tohoku University School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980, Japan

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Summary

Structural changes of guinea pig taenia coli and rat anococcygeus muscle during contraction were studied by X-ray diffraction. The diffraction pattern of the taenia coli showed the 14.4-nm myosin reflection, the 5.9-nm actin layer-line and a diffuse equatorial peak at 1/11.4 nm⁻¹. On application of carbachol, the muscle contracted and the intensity of the 14.4-nm reflection showed a concentration-dependent decrease: the maximum decrease was 24% at 2×10⁻⁵ M. Such an intensity decrease was not observed in K-contracture (154 mM). The intensity of the 5.9-nm actin layer-line did not change appreciably on activation. The equatorial peak became broader during contraction. The 14.4-nm myosin reflection of the anococcygeus muscle was weak. Its intensity increased by 106% during contraction induced by 2×10⁻⁵M phenylephrine and by 75% during K-contracture. These results suggest that the number of myosin filaments may increase during contraction of rat anococcygeus muscle but not guinea pig taenia coli.

Introduction

X-ray diffraction studies on mammalian smooth muscles were pioneered by Jack Lowy and his colleagues (Elliott & Lowy, 1968). They observed reflections from both thick and thin filaments (Lowy et al., 1970) and found intensification of some of the actin layer-lines in the activated state which suggested a shift of tropomyosin on the thin filament (Vibert et al., 1972).

Since the work of Lowy and colleagues, plenty of findings have been published on the molecular mechanism of smooth muscle contraction and its regulation. The phosphorylation of the myosin light chain is now regarded as a major regulatory mechanism (Kamm & Stull, 1985), although there may be additional regulatory systems linked to the thin filament (Sobue et al., 1981; Marston & Lehman, 1985; Takahashi et al., 1986; Ebashi, 1989). It has been demonstrated that the polymerization of a myosin molecule from smooth muscle also depends on phosphorylation of its regulatory light chain (Suzuki et al., 1978; Craig et al., 1983). When the regulatory light chain is unphosphorylated, the tail portion of the myosin binds to its head–tail junction and the myosin does not readily polymerize. When it is phosphorylated, the myosin is straighter and polymerizes into filaments (Onishi & Wakabayashi, 1982; Trybus & Lowey, 1984). However, this phenomenon has not been confirmed in intact smooth muscle cells.

The present experiments were made to study the structural changes associated with smooth muscle contraction in the light of current knowledge, using an intense X-ray beam from a synchrotron radiation source. To study the polymerization of myosin into filaments, two different muscles were studied: the taenia coli of the guinea pig and the anococcygeus muscle of the rat. These muscles have been reported to be different in their regulation of myosin polymerization when studied by optical birefringence (Godfraind-De Becker & Gillis, 1988).

Materials and methods

Specimen preparation

A taenia coli, about 1 cm in length and 1–2 mm in width, was dissected from a guinea pig killed by an overdose of pentobarbital. To avoid excessive contraction during dissection, it was relaxed in situ with 5×10⁻⁶ M adrenaline and its length was measured. After dissection, the muscle was transferred to a specimen chamber and its ends were tied to hooks with threads. One hook was connected to a strain gauge tension transducer (UL-
with a specimen-to-plate distance of 110-250 cm. The Photon Factory, Tsukuba, using the small-angle X-ray Tokyo) using a program written by N. Yagi. Fuji Film, Tokyo). The diffraction patterns were analysed were scanned by the image reader described by Amemiya even after six exposures on the same specimen. The plates radiation damage was noted in the diffraction pattern was contracted several times, successive contractions in the two control exposures. When the same specimen compared with that obtained by averaging the intensities muscle in a relaxed state before and after each contraction 14.4-nm myosin reflection, was observed after prolonged was returned to the normal external solution whereas the anococcygeus muscle was contracted by 2x10^-6 M phenylephrine or the high-K solution. Exposure to these agonists and the high-K solution was kept shorter than 5 min because irreversible changes in the diffraction pattern, such as a large drop in the intensity of the 14.4-nm myosin reflection, was observed after prolonged contraction. The anococcygeus muscle relaxed when it was returned to the normal external solution whereas the taenia coli contracted spontaneously and was therefore relaxed with 5x10^-6 M adrenaline. After the muscle relaxed, the bathing solution was changed to the normal external solution. Control exposures were made with the muscle in a relaxed state before and after each contraction and the intensity of the reflections during contraction was compared with that obtained by averaging the intensities in the two control exposures. When the same specimen was contracted several times, successive contractions were made at intervals of more than 20 min.

X-ray techniques and data analysis

X-ray experiments were made at the beam line 15A in Photon Factory, Tsukuba, using the small-angle X-ray camera described by Amemiya and colleagues (1983). Diffraction patterns were recorded on Fuji imaging plates with a specimen-to-plate distance of 110-250 cm. The size of the beam on the specimen was matched to the size of the specimen. The exposure time was 30 s. No radiation damage was noted in the diffraction pattern even after six exposures on the same specimen. The plates were scanned by the image reader described by Amemiya and colleagues (1987) or a commercial scanner (BAS2000, Fuji Film, Tokyo). The diffraction patterns were analysed on an engineering workstation (NEWS-3460, Sony, Tokyo) using a program written by N. Yagi.

The spacings of the reflections were calibrated by using the 14.3-nm meridional reflection in a resting frog sartorius muscle at 1/14.34 nm^-1 (Haselgrove, 1975). The meridional reflections appeared as arcs of 10-20 degrees (Fig. 1a-c). Thus the intensity distribution along the meridian was obtained by averaging intensities at a fixed distance from the origin within the arc of 30 degrees across the meridian. The equatorial profile was similarly obtained. The axial intensity profile of the 5.9-nm actin layer-line was obtained by integrating intensities in the lateral region of 0.037-0.160 nm^-1. To quantify the intensity of a reflection, a straight line was drawn by connecting the background points on each side of the reflection and the area above the line was measured.

Special care was taken to measure the intensity of the 14.4-nm myosin meridional reflection from the rat anococcygeus muscle, which is just above the noise level (Fig. 2c and d). The patterns were randomly numbered and the intensities were measured without any information on the state of the specimen. The background was drawn in by eye and the area above the background was measured. The results obtained were not significantly different from those obtained by drawing a linear background as was done for other reflections.

The intensities were normalized by the total intensity in the diffraction pattern (after subtraction of the air scatter). The data from a specimen was discarded if the total intensity varied by more than 20% between exposures.

The intensity profile of the equatorial reflection between 0.05 and 0.13 nm^-1 was fitted by the sum of one Gaussian peak and two Gaussian background functions using a curve fitting programme SALS on ACOS2020 at Tohoku University Computer Center.

Throughout this paper, the statistics are shown as a mean ± standard error of the mean.

Results

GUINEA PIG TAENIA COLI

Resting state

Three main features were observed in the X-ray diffraction pattern from guinea pig taenia coli in a resting state (Fig. 1a), as previously reported by Lowy and colleagues (1970). The presence of 5x10^-6 M adrenaline did not affect these features.

The third, fifth and higher order meridional reflections from collagen were observed (Fig. 2a). The Bragg spacing of the fifth reflection was 13.02 ± 0.01 nm (n = 4). The meridional reflection from the thick filament was seen at a Bragg spacing of 14.42 ± 0.1 nm (n = 7). An intensity peak was found at 1/7.2 nm^-1 which may be regarded as the second order myosin reflection but it was too close to the ninth order reflection of collagen (at 1/7.23 nm^-1) to define its origin. These reflections were observed as arcs across the meridian with a width at half-maximum of 10-20 degrees. The arcs indicate that the collagen fibres