Adrenergic innervation and contractile activity of the mesotubarium superius of the rabbit oviduct

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Summary. Various patterns of spontaneous contractile activity were observed in the mesotubarium in vitro, but generally the frequency was lower and the amplitude was higher in the contractions than in those of the spontaneously motile oviduct. Contractile responses to norepinephrine occurred at concentrations higher than 10^{-8} M. Twitch-like contractile responses were produced by field stimulation of 1 msec, 1-30 Hz and 100 V for 10 sec. Responses to norepinephrine and electrical stimulation were inhibited by phentolamine or phenoxybenzamine, but were not affected by propranolol. Spontaneous contractile activity of the mesotubarium was diminished but the tonus was not affected by isoproterenol. These results suggest that the responses of the mesotubarium to norepinephrine and electrical stimulation are mainly related to the α-receptor rather than to the β-receptor component.

Introduction

The mesotubarium is the membranous smooth muscle layer located along the oviduct which connects the fimbrial and uterine ends. It has been proposed that contraction of this muscle may influence either the location of the fimbria relative to the ovary during ovulation (Blandau & Verdugo, 1976) or the contraction pattern of the oviduct (Gimeno, Rettori, Gimeno & Coutinho, 1974). By means of catecholamine histochemistry and the mechanical contractile response to norepinephrine, we have demonstrated the presence of an adrenergic innervation of the rabbit mesotubarium (Doteuchi, 1976). Halbert & Conrad (1975) have described various patterns of spontaneous contractile activity of rabbit mesotubarium under various hormonal conditions, and Meiss (1975) analysed the contractile response of this muscle to electrical stimulation. In the present study, we investigated the mesotubarial response to catecholamines and electrical stimulation in vitro, compared the response to that of oviduct, and tested the effects of α- and β-adrenoreceptor blockers on these responses.

Materials and Methods

Mature non-pregnant female rabbits were kept in individual cages and isolated from males for at least 2 weeks. After the animals had been killed by cervical dislocation, the entire reproductive tract including the ovaries, oviducts, uterus and associated ligaments was removed and washed with Krebs’ bicarbonate buffer. The mesotubarium, dissected into about five 15-mm pieces as indicated in Pl. 1, Fig. 1, was mounted in a Magnus apparatus containing 30 ml Krebs’ bicarbonate buffer bubbled with 95% O_2 + 5% CO_2 kept at 37°C. Contraction was isometrically recorded by a force displacement transducer (NIHON KOHDEN SB-1T) connected to a polygraph (NIHON KOHDEN RM-150). An isthmic segment of oviduct about 1·5 cm long was carefully removed from the mesotubarium and mesosalpinx, and one end was ligated. Contraction of the longitudinal muscle was recorded as for the mesotubarium and that of the circular muscle was registered as the change in the intraluminal pressure of the tube from an open-ended cannula by a pressure transducer (NIHON KOHDEN MP-24S) connected to the polygraph. Resting tensions of 250 and 500 mg were applied to the mesotubarium and oviduct longitudinal muscle respectively: the tension in each preparation slightly decreased

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(10–15%) before the appearance of spontaneous contractions, after which the tension was maintained. For circular muscle measurements, an intraluminal pressure of 40 mmH₂O was initially applied but was decreased about 30% before the tests.

Electrical stimulation (1 and 10 msec, 100 V at various frequencies for 10 sec) was applied, at 7- to 10-min intervals, through the electrodes of a platinum-wire ring placed on either side of the tissue by an electrical stimulator (NIHON KOHDEN MSE-30).

Histochemical preparations for catecholamines were prepared by freeze-drying the tissues stretched on glass slides, followed by exposure to paraformaldehyde gas at 80°C for 1 h. The sectioned preparations were examined with a Zeiss standard fluorescence microscope RA-38 with emission filter BG 12 and barrier filter OG 4 as described by Falck & Owman (1965). Histological preparations were made by stretching the tissues on glass slides followed by fixation with 10% formalin and haematoxylin–eosin staining.

The effects of the following drugs were tested: phenoxybenzamine hydrochloride, phentolamine mesylate, propranolol hydrochloride, norepinephrine bitartrate, and isoproterenol hydrochloride. All drugs were dissolved in 0·15 M-NaCl, except phenoxybenzamine which was dissolved first in a small amount of ethanol then diluted with 0·15 M-NaCl. All drug solutions were prepared fresh daily and kept on ice during the experiments.

Results

Adrenergic innervation

As shown in Pl. 1, Fig. 1, a number of thin bundles of smooth muscle can be seen in the mesotubarium superius as well as in the mesosalpinx. Some run parallel with and others are at right angles to the oviduct. Adrenergic nerve fibres with strong catecholamine fluorescence were present in the mesotubarium (Pl. 1, Fig. 2). Almost all fluorescent fibres seemed to be associated with the smooth muscle cells of the mesotubarium as they were in the same direction and in close contact with them. Some of the fluorescent fibres were in close contact with blood vessels. The extent of adrenergic innervation in this part of the mesotubarium was less than that in the circular muscle layer of the isthmus (Pl. 1, Fig. 3), and probably more than that in the longitudinal muscle layer. However, in that part of the mesotubarium which is surrounded by the major arch of the ampulla, nerve fibres with catecholamine fluorescence were scarce.

Spontaneous contractile activity

Mesotubaria from different animals showed various patterns of spontaneous contractile activity as shown in Text-fig. 1, but each animal exhibited a characteristic pattern which persisted almost invariably throughout the observation period. The spontaneous contractile activity could be grouped into three typical patterns: (a) high-frequency (1·5–3/min) and relatively low-amplitude phasic contractions (Text-fig. 1a); (b) low-frequency (less than 0·5/min) regular contractions of high amplitude, (Text-fig. 1b); (c) tonic contractions lasting 3–5 min appearing at 3- to 5-min intervals, often superimposed with high-frequency phasic contractions (Text-fig. 1c). Sometimes a mixed pattern of (b) and (c), as shown in Text-fig. 1(d), was observed. Pattern (b) was the most frequently observed pattern in the present experiments. In all cases, spontaneous contractions appeared following a quiet period of 15–30 min after the tissues had been placed in the apparatus and lasted for 1·5–2 h under the experimental conditions used.

Typical patterns of spontaneous contraction of the mesotubarium and longitudinal and circular muscles of the oviduct from the same animal were compared (Text-fig. 2). Generally, the frequency of spontaneous contraction of the mesotubarium was lower than those of the longitudinal and circular muscles. In contrast, the amplitude of the mesotubarial contractions was considerably higher than that of the longitudinal muscle.
Text-fig. 1. Various patterns of spontaneous contractile activity of mesotubaria isolated from the oviducts of different rabbits at normal oestrus.

Text-fig. 2. Comparison of the spontaneous contractile activity of the mesotubarium (M), longitudinal (L) and circular (C) muscles of the oviduct from the same rabbit. Note that the frequency of the mesotubarial contractions is considerably less than those of the muscles.

Response to chemical and electrical stimulation

Because it was difficult to estimate the effect of stimulation when the response was superimposed on the spontaneous contraction, norepinephrine and electrical stimulation were applied after the spontaneous activity had weakened. Text-figure 3 shows the typical contractile response of the mesotubarium and the longitudinal and circular muscles of the oviduct to norepinephrine. In most cases,
**Text-fig. 3.** Comparison of the contractile responses of mesotubarium (M), longitudinal (L) and circular (C) muscles of the oviduct from the same rabbit to various concentrations of norepinephrine (NE). The drug was not washed out until the tonus returned to the basal level.

**Text-fig. 4.** Effects of α- and β-blockers on the responses of the rabbit mesotubarium to norepinephrine (NE) and isoproterenol (ISO) tested 30 min after the application of drugs. W = wash.
Fig. 1. Rabbit mesotubarium superius and oviduct stretched on a glass slide and stained with haematoxylin-eosin. A, ampulla; I, isthmus; M, mesotubarium superius; Mx, mesosalpinx; U, uterus; F, fimbrìa. Horizontal bar indicates 1 cm. The dashed rectangle indicates the area taken for experiments.

Figs. 2 and 3. Adrenergic nerves with catecholamine fluorescence in the mesotubarium superius isolated from the isthmic portion of the oviduct (Fig. 2) and in the isthmus of the oviduct (Fig. 3). Most of the fluorescent nerves with varicosities run along smooth muscle cells and some (arrowed) are associated with the blood vessels. Horizontal bar indicates 100 μm.

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the mesotubarium responded to a lower concentration of the drug than did the longitudinal and circular muscles. The contraction pattern of the mesotubarium induced by norepinephrine was also different from those of the longitudinal and circular muscles.

The minimum effective norepinephrine concentration producing contraction of the mesotubarium was $10^{-8}\text{M}$ for 5 samples and $5 \times 10^{-8}\text{M}$ for a sixth sample: the amplitude of the contractions slightly increased and the duration became longer with the higher concentrations. For the oviductal longitudinal and circular muscles, the minimum effective concentration of norepinephrine was $5 \times 10^{-8}\text{M}$

Text-fig. 5. Contractile response of the rabbit mesotubarium to electrical stimulation (a) and the relationship between stimulation frequency and amplitude of the response (b). Each point shows the mean ± s.e.m. of at least 4 observations.

Text-fig. 6. Effects of phentolamine, phenoxybenzamine and propranolol on the contractile responses of rabbit mesotubarium to electrical stimulation. The solid circles (●) indicate the stimulation with a pulse of 1 msec duration (30 Hz and 100 V for 10 sec) and the open circles (○) indicate the stimulation with a pulse of 10 msec duration (30 Hz and 100 V for 10 sec). Note the appearance of contractions, independent of stimulation, after application of the α-blockers.
for 2 samples, $10^{-7}$ M for 3 samples and $3 \times 10^{-6}$ M for the sixth; both amplitude and duration of the phasic contraction increased with increasing drug concentration. Also, in most cases, there was a superimposed high-frequency phasic contraction. The contractile responses of the mesotubarium to $5 \times 10^{-8}$ to $10^{-6}$ M norepinephrine were completely blocked 30 min after application of either $3 \times 10^{-6}$ M-phentolamine or $10^{-5}$ M-phenoxybenzamine (Text-fig. 4).

Concentrations of isoproterenol of more than $10^{-8}$ M diminished the spontaneous contractions of the mesotubarium, but there was no decrease in basal muscle tone with concentrations of $10^{-8}$ to $10^{-5}$ M. Slight relaxation of the mesotubarium was sometimes produced by $10^{-4}$ M-isoproterenol and this response was blocked by pretreatment with $10^{-5}$ M-propranolol (Text-fig. 4).

Contractile responses to electrical stimulation were produced in all preparations of mesotubarium. As shown in Text-fig. 5, the amplitude of the contractile response increased with the increase of stimulation frequency up to 30 Hz where the maximum contraction was obtained. The amplitude of the contractile response to electrical stimulation of 1 msec, 30 Hz and 100 V for 10 sec was markedly diminished by pretreatment with $3 \times 10^{-6}$ M-phentolamine or $10^{-5}$ M-phenoxybenzamine (Text-fig. 6), but the response to electrical stimulation of 10 msec, 30 Hz and 100 V for 10 sec was not affected by this pretreatment.

**Discussion**

Halbert & Conrad (1975) have demonstrated various patterns of spontaneous contractile activity of the mesotubarium superius of the rabbit oviduct in vitro in various hormonal conditions. In the present study, also with rabbit mesotubarium, we observed various patterns of spontaneous contraction even though all the samples were from oestrous animals. Although Halbert & Conrad (1975) state that the mesotubarium has a geometrically simple structure which facilitates analysis of its contractile activity, this is true only of the part near its free margin which runs along the isthmic portion of the oviduct. In the other parts, for instance those surrounded by the major arch of the ampulla, the direction of muscle fibres is not always parallel, and many fibres traverse the major bundle (Pl. 1, Fig. 1). The diversity of the spontaneous contractile activity of the mesotubarium seems to be at least partly due to the mixing of muscle fibres in different directions.

Brundin, Fredricsson, Norberg & Swedin (1969) have briefly reported the presence of adrenergic innervation in rat mesotubarium. The influence of the broad ligament on the contractile activity of the oviduct has been indicated by Gimeno et al. (1974) who studied isolated guinea-pig organs. It is difficult to distinguish and separate the mesotubarium and mesosalpinx in guinea-pigs because of the coiled shape of the oviduct. In the rabbit, however, two thin layers of smooth muscle can be clearly seen on both sides of the oviduct; one is the mesosalpinx attached to the body wall and the other is the mesotubarium superius with its free margin floating in the body cavity and the influences of the mesosalpinx and the mesotubarium on the contraction of the oviduct and ovum transport may be different. This was why we first studied the pharmacological responses of the mesotubarium.

The adrenergic innervation of the mesotubarium in rabbits appeared to be essentially similar to that of the oviduct, as reported by Brundin & Wirsen (1964), Rosengren & Sjöberg (1968) and Takeda & Doteuchi (1976), in that most of the fluorescent nerve fibres run along the smooth muscle cells, although some are distributed to the blood vessels, and that the number of adrenergic nerves was much greater in the circular muscle layer than in the mesotubarium. In rats (Brundín et al., 1969) and guinea-pigs (unpublished observations), however, the number of nerves with catecholamine fluorescence in the circular muscle is less than that in the mesotubarium. Johns & Paton (1976) interpreted the higher sensitivity of the longitudinal than of the circular muscle layer to norepinephrine in the ampulla of the rabbit as being due to a greater concentration of adrenergic nerves in the latter, but we found no difference in the sensitivity of these muscle layers of the isthmus, despite the greater innervation of the circular muscle layer. Furthermore, the difference in the sensitivity of the circular muscle and the mesotubarium cannot be explained by the hypothesis of Johns & Paton (1976). Qualitative and quantitative similarity have been shown in the response to α-adrenoreceptor stimulation with phenylephrine in the isthmus and the ampulla of rabbit oviduct (Levy & Lindner, 1972), though the adrenergic nerves in the ampulla are few and almost exclusively found in perivascular areas (Brundin...
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& Wirsen, 1964). One of the characteristic patterns of the mesotubarium response to norepinephrine was abrupt termination of tonic contractions in the presence of the agonist in the bath fluid. The reason is not clear at the present time; we do not consider that it can be fully explained by inactivation of the drug by uptake into the nerves or by enzymatic degradation in the tissue, as the amount of norepinephrine in the bath fluid (30 ml) should have been adequate for the amount of tissue used (5–7 mg wet weight).

The contractile response of the mesotubarium to norepinephrine was completely blocked by phenoxybenzamine and phentolamine, but was not reversed to give relaxation as reported for the human oviduct (Nakanishi, Wansbrough & Wood, 1967). This suggests that in rabbit mesotubarium the β-adrenoceptor activity to relax the muscle is not as high as that in the human oviduct. Our results with isoproterenol support this; a high concentration of isoproterenol was required to relax the mesotubarium, while relatively low concentrations diminished the spontaneous contractile activity. Similar results were also obtained in experiments with rabbit mesotubarium in vivo (Doteuchi, Otani & Takeda, 1976).

Maximum contraction occurs with electrical stimulation at a frequency of 16–20 Hz in longitudinal and circular muscles of rabbit oviduct (Johns & Paton, 1975) and at 25 Hz in the mesotubarium (Meiss, 1975). We obtained similar results in the present study. Phentolamine or phenoxybenzamine almost completely blocked contractions produced by electrical stimulation with a 1-msec pulse duration but did not affect the response to stimulation with a pulse of longer duration. These findings suggest that the contractile response of the mesotubarium to electrical stimulation with 1-msec pulses is due to norepinephrine released from adrenergic nerve terminals acting on the α-adrenoceptor which is predominant in this preparation, as similarly demonstrated for the oviduct (Paton, 1976). β-Adrenoceptor activity in the mesotubarium is considered to be less important in the contractile response or in maintaining the tonus of the muscles since the response to electrical stimulation was not affected by the β-blocker propranolol.

References


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