Praca oryginalna *Original paper*

Gap junction proteins in nonpregnant porcine myometrium*)

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Summary

The aim of this study was to analyse the expression of gap junction proteins, connexins, in non-pregnant porcine myometrium. Uterine tissue was obtained immediately after slaughter of the animals and the examination of ovarian morphology. Tissues were collected from prepubertal and mature pigs at preovulatory and secretory phases of the oestrous cycle and frozen in liquid nitrogen. Cryosections were immunofluorescently labelled using antibodies against connexins 26, 32, 40 and 43. Among four connexins studied, only connexin 43 was detected in the myometrium of the immature and adult porcine uterus. Connexin 43 labelling appeared as bright fluorescent spots distributed along smooth muscle cell interfaces. The amount of labelling for Cx43 was much higher in the circular layer than in the longitudinal layer in all prepubertal and mature porcine myometria. These results support the concept that connexin 43 is the principal connexin expressed in the nonpregnant myometrium. Furthermore, it seems that muscle layer-specific distribution of connexin 43 gap junctions may contribute to diverse functions of circular and longitudinal smooth muscles in modulating uterine motility.

Keywords: connexin, myometrium, pig

Uterine muscles play an important role through changes in their contraction-relaxation cycle in the coordination of uterine activities during defined physiological events of reproduction (8). Spontaneous uterine motility of varied frequency and amplitude occurs throughout the oestrous cycle (14). During most of pregnancy, uterine myometrium is relatively quiescent while the most powerful contractions develop at the onset of labour. At parturition the uterus is highly responsive to various uterotonins and synchronous contractions facilitate successful delivery of foetus (8). The mechanisms that regulate contractility in uterus during oestrous cycle, pregnancy and labour are only partially understood. An important aspect of this regulation is cell-to-cell communication through myometrial gap junctions. Gap junctions are transmembrane channels that allow interchange of signalling molecules, flow of current between adjacent cells and mechanical coupling with gap junction interacting proteins (7, 17). According to Moore and Burt (15) each gap junction is composed of two hexamers of proteins termed connexins. The principle protein of myometrial gap junctions is connexin 43 (Cx43) and further gap junctional proteins identified in human myometrium at term are Cx40 and Cx45 (11). Gap junctions are formed rapidly and in large numbers within a few hours before parturition and they come to occupy 0.2-0.4% of the cell surface (5). Furthermore, in rodents progesterone suppresses the formation of myometrial gap junctions, whereas estrogen promotes it (16). The number and/or size of gap junction plaques increase progressively as electrical and metabolite coupling improve and decrease in parallel with a loss of communication (2). Therefore, during pregnancy is an input resistance at gap junctions, but at term, more pronounced is input conductivity. This and other evidence implicates gap junctions as the morphological correlate of the low-resistance cell-to-cell pathway (2, 16, 17). Taken together, it is generally accepted that the rapid formation of gap junctions in the myometrium contributes to the termination of pregnancy by synchronizing contractions of individual smooth muscle cells under hormonal control (5, 6, 8, 11, 16). However, little is known about gap junction proteins in the nonpregnant myometrium. In nonpregnant human and rodents, Cx43 gap junctions are present at low frequency and are small in size (11, 16) but strong and abundant signal of Cx43 labelling was reported in nonpregnant porcine myometrium (10, 18). Hence, porcine uterus may serve as a suitable model to study protein composition and distribution of gap junctions in uterine smooth muscles at various physiological conditions.

The aim of this study was to analyse the distribution of Cx43 in the myometrium of prepubertal and mature

*) This work was supported by KBN grant 3 P06K 030 24.

pigs at different stages of the oestrous cycle and to examine whether other gap junction proteins Cx26, Cx32 and Cx40 are expressed in porcine myometrium.

Material and methods

Animals and tissue preparation. Nonpregnant porcine uteri were collected immediately after slaughter of the animals from the local slaughterhouse. Uterine samples were taken from 3 prepubertal and 6 mature animals at preovulatory (n = 3) and secretory (luteal) (n = 3) phases after examination of ovarian morphology. Samples of porcine ventricular myocardium and liver, and foetal mouse muscles were used as control tissues. All samples were frozen immediately in liquid nitrogen for cryosectioning.

Antibodies. Monoclonal antibodies against Cx26 and Cx32 were purchased from Zymed (San Francisco). Polyclonal antibody against Cx40 was a generous gift from Professor Nicholas J. Severs (National Heart and Lung Institute, Imperial College School of Medicine, UK). Rabbit polyclonal antibody against Cx43 was purchased from Sigma. The fluorescent secondary antibodies used were goat anti-mouse Cy3 and goat anti-rabbit Cy3-conjugated (Jackson Immunoresearch).

Immunofluorescent labelling. Frozen sections (10 μm) of porcine uterus, heart and liver, and foetal mouse muscles were cut in a cryomicrotome (Shandon Scientific Ltd., UK) and mounted on gelatine coated glass slides. The sections were fixed in 2% paraformaldehyde in PBS and than incubated in blocking solution of 5% horse serum and 5% goat serum for 1 hour. Primary antibody against Cx26 (diluted 1 : 250 in blocking solution), Cx32 (1 : 500), Cx40 (1 : 500) and Cx43 (1 : 2000) was applied overnight at room temperature. After washing with PBS the sections were blocked for 1 hour and incubated for another hour with appropriate secondary antibody. The slides were washed in PBS and mounted in propyl galate medium. Control experiments were carried out in which the primary antibody was omitted. Immunolabeled sections were examined by Nikon light microscopy (Nikon, Japan) equipped with epifluorescence and filter for maximum Cy3 fluorescence at objective magnification 40 × and numerical aperture 0.75. Images were captured in digital format using Nikon digital camera DXM 1200F.

Results and discussion

The specificity of the labelling for the detection of Cx43 gap junctions was confirmed using porcine cardiac muscle as a positive control (fig. 1a). Connexin 43 gap junctions were detectable in the myometrium as clearly defined fluorescent spots at the smooth muscle cell outlines (fig. 1b, c). There was prominent labelling for Cx43 in the circular layer and only weak labelling in the longitudinal layer in all specimens from immature and mature porcine myometrium. Evaluation of specimens obtained from mature porcine uteri revealed that Cx43 gap junctions were abundant in preovulatory myometrium but less numerous at secretory (luteal) phase of the oestrous cycle (not shown). Connexin 40 was clearly detected in foetal mouse muscles while Cx32 and Cx26 were observed in porcine liver, which were used as positive controls (fig. 1d, g, j). However, no labelling for Cx40, Cx32 and Cx26 was found in the examined myometria (fig. 1e, f, h, i, k, l). Controls with primary antibodies omitted were negative (not shown).

The principal finding of this study is that Cx43 is expressed differentially in the circular and longitudinal layers of the nonpregnant porcine myometrium. To our knowledge this is the first report of diverse distribution of Cx43 gap junctions in the porcine myo-

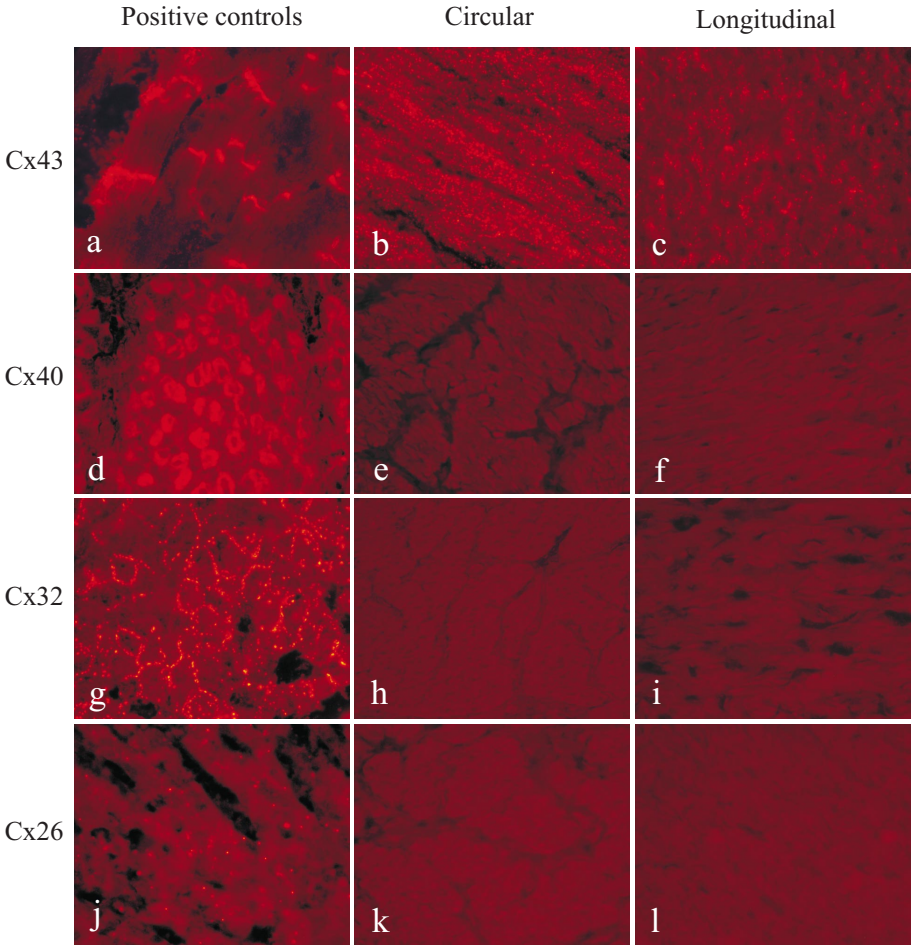

Fig. 1. Immunofluorescent localization of Cx43, Cx40, Cx32 and Cx26 in preovulatory porcine myometrium and control tissues. Prominent labelling for Cx43 gap junctions is seen within intercalated discs of cardiomyocytes (a) and in the circular layer of porcine myometrium (b). Only week labelling for Cx43 occurs in the longitudinal layer (c). Intense labelling for Cx40, Cx32 and Cx26 was evident in control tissues: foetal mouse muscles (d) and porcine liver (g, j). However, no labelling for Cx40, Cx32 and Cx26 was detected in the porcine myometrium (e, f, h, i, k, l)

metrium. Furthermore, connexin26, Cx32 and Cx40 were not detected in prepubertal or mature myometrium supporting the notion that Cx43 is the major connexin expressed in the nonpregnant uterine smooth muscles.

In humans and other mammals, gap junctions are scarce in the myometrium of nonpregnant uterus. However, Cx43 may be readily detected with immunofluorescence and Western blotting in the nonpregnant porcine myometrium (10, 18). Our present results are in agreement with these reports. Hence, the porcine uterus may provide attractive model for studying mechanisms triggering expression of gap junction proteins in the cycling myometrium. There was striking difference in Cx43 expression between the circular and longitudinal layers of immature and cycling porcine myometrium. Similar results were reported for the nonpregnant bovine and rabbit (4) and immature rat treated with oestrogen (16). Therefore, myometrial Cx43 gap junctions in porcine uterus fits the general principle that incomparably lesser extend of gap junction distribution takes place in longitudinal muscle than in the adjacent circular muscle. These data collectively demonstrate that Cx43 is regulated differentially in myocytes from the circular and longitudinal myometrium. The myometrial layers have different embryological origin and physiological characteristics. The contractions of the circular muscle layer constrict uterine lumen and those of the longitudinal muscle layer shorten uterine horns (3). Strong contraction of the longitudinal muscle might be required for transport of luminal contents, but relaxation, not contraction, of the circular muscle is required for preservation of luminal contents (1). In the porcine uterus, smooth muscle layer-dependent differences were demonstrated in the autonomic innervations, the distribution of oxytocin, muscarinic, histamine and endothelin receptors, and in mechanical responses to various contractile agents (acetylcholine, norepinephrine, histamine, oxytocin and endothelin) (1, 9, 12). Moreover, sensitivity to contractile factors is higher in the longitudinal muscle than in the circular muscle layer whereas the opposite is true for the relaxing factors (1, 12, 13). This diversity was assumed to reflect the different function of longitudinal and circular muscles in uterine motility. In fact, it has been reported that isolated myometrial strips of the nonpregnant porcine uterus contract spontaneously in Krebs solution, and the frequency of the contraction in the circular muscle is significantly higher than that in the longitudinal muscle (1, 13). Our finding that Cx43 gap junction protein is expressed at higher level in the circular than in longitudinal layer of the porcine myometrium is consistent with evidence of different mechanical activity of the circular and longitudinal porcine myometrium reported by other authors (1, 13). This would imply that diverse distribution of gap junctions in the myometrium might be involved in regulation of contractile functions of smooth muscles in non-pregnant uterus. However, mechanism(s) responsible for diverse expression of gap junctions is not yet known. Taken together, these results indicate that the regulation of myometrium is complex and the final pattern of contractile activity involves both muscle layers.

In the present study, we have found that expression of Cx43 gap junctions in porcine myometrium changes during oestrous cycle in accordance with previous report by Thilander et al. (18). These results suggest that steroid hormones, progesterone and/or oestradiol, may be involved in regulation of Cx43 expression during the oestrous cycle. In cycling pigs, spontaneous myometrial activity increases during oestrus and stimulation or suppression of uterine contractility may influence the transport of sperm cells through the horns (14). Since cell-to-cell communication mediated through gap junctions is essential to the synchronization of contractions in uterine muscles it is possible that changes in Cx43 expression in cycling myometrium may contribute to modulation of uterine wall tonus and motility during oestrous cycle.

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