Properties of Spontaneous Electrical Activity in Smooth Muscle of the Guinea-Pig Renal Pelvis

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Abstract: In the guinea-pig renal pelvis, most smooth muscle cells examined (>90%), using a conventional microelectrode, had a resting membrane potential of about −50 mV and produced spontaneous action potentials with initial fast spikes and following plateau potentials. The remainder (<10%) had a resting membrane potential of about −40 mV and produced periodical depolarization with slow rising and falling phases. Experiments were carried out to investigate the properties of spontaneous action potentials. The potentials were abolished by nifedipine, suggesting a possible contribution of voltage-gated Ca^{2+} channels to the generation of these potentials. Niflumic acid and 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), inhibitors of Ca^{2+}-activated Cl⁻ channels, showed different effects on the spontaneous action potentials, and the former but not the latter inhibited the activities, raised the question of an involvement of Cl⁻ channels in the generation of these activities. Depleting internal Ca^{2+} stores directly with caffeine or indirectly by inhibiting Ca^{2+}-ATPase at the internal membrane with cyclopiazonic acid (CPA) prevented the generation of spontaneous activity. Chelating intracellular Ca^{2+} by 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) increased the amplitude of the spike component of spontaneous activity. Indomethacin inhibited the spontaneous activity, whereas prostaglandin F_{2α} enhanced it. The results indicate that in smooth muscle of the renal pelvis, the generation of spontaneous activity is causally related to the activation of voltage-gated Ca^{2+} channels through which the influx of Ca^{2+} may trigger the release of Ca^{2+} from the internal stores to activate a set of ion channels at the membrane. Endogenous prostaglandins may be involved in the initiation of spontaneous activity.

Key words: renal pelvis, electrical activity, plateau potential, voltage-sensitive Ca^{2+} channel, internal Ca^{2+} store.

Smooth muscle of the upper urinary tract is spontaneously active with rhythmic contraction, and the pacemaker cells that mediate the activity are suggested to be in the distal region of the calyces [1]. The propagation of rhythmic contractions along the ureter allows urine to be transported to the bladder. In a cat, the conduction velocity of muscle excitation changes at the pelvic-ureter border, suggesting that the electrical properties of pelvis smooth muscles may differ from those of ureter muscles [2]. The spontaneous activities appearing in the renal pelvis are more sensitive to Ca^{2+} than Na^{+} is, suggesting that they are myogenic in origin [3]. The renal pelvis consists of two types of smooth muscle cells, “typical” and “atypical” cells, both distributed in the renal calyx and pelvi-calyceal junction regions, and the atypical cells are suggested as the “pacemaker” cells [4–6]. Electrical responses recorded in pelvic smooth muscles of the rabbit [7] and guinea-pig [8, 9] reveal that the potentials form either slow rising and falling changes or an initial spike and that they follow plateau components. Staining recorded cells with neurobiotin...
or lucifer yellow confirmed that the typical smooth muscle cells generate action potentials with an initial spike and following plateau components, whereas the atypical smooth muscle cells generate pacemaker potentials with slow rising and falling phases [6]. However, the cellular mechanisms, including ionic mechanisms of the generation of spontaneous activity in smooth muscle of the renal pelvis, remain unclear.

We investigated the properties of spontaneous activity in smooth muscle of the guinea-pig renal pelvis. The activities are considered myogenic, since they are not inhibited by tetrodotoxin [8–11]. The experiments were carried out to observe the effects of the modulation of cellular functions through Ca\(^{2+}\)-dependent processes in smooth muscle cells on spontaneous activities recorded from the pelvic smooth muscle cells. The following chemicals were tested: nifedipine, an inhibitor of voltage-gated L-type Ca\(^{2+}\) channels; niflumic acid and DIDS, inhibitors of Ca\(^{2+}\)-activated Cl\(^{-}\) channels [12]; BAPTA, a chelator of intracellular Ca\(^{2+}\) [13]; caffeine, a depletor of Ca\(^{2+}\) from the internal stores [14, 15]; and cyclopiazonic acid, an inhibitor of Ca\(^{2+}\)-ATPase at the internal Ca\(^{2+}\) store [16].

The activity appearing in the pelvic smooth muscle is reportedly inhibited by indomethacin, an inhibitor of cyclooxygenase, suggesting that it is generated through the production of endogenous prostaglandins, possibly prostaglandin F\(_{2a}\) (PGF\(_{2a}\)) [9]. The effects of inhibitors of cyclooxygenase, indomethacin, and PGF\(_{2a}\) on spontaneous activity were also observed in the pelvic smooth muscle.

**METHODS**

Male guinea-pigs, weighing 200 to 300 g, were anesthetized with diethyl ether and exsanguinated. The protocols used conformed with guidelines on the conduct of animal experiments issued by the government and were approved by the Committee on the Ethics of Animal Experiments in Nagoya City University Medical School. The kidney was isolated and hemisected with a razor. The smooth muscle tissues including renal calyx, renal pelvis, and a part of the upper ureter were carefully isolated from the parenchyma in Krebs solution at room temperature. The adipose tissues, connective tissues, and mucous membrane were removed from the smooth muscle tissue under a binocular dissecting microscope. The tissue segment was mounted with the mucosal side up on a silicon rubber plate fixed at the bottom of the recording chamber (capacity of 2 ml), which was made of acrylic plastic. The tissue was immobilized by tiny pins and was superfused with warmed (35°C) Krebs solution at a constant flow rate of 2 to 3 ml min\(^{-1}\).

Electrical responses of smooth muscle cell were recorded from the proximal renal pelvic region (3 mm width, 2 mm length), using glass capillary microelectrodes made of borosilicate glass tube (1.2 mm OD) with a fine inner filament (Hilgenberg, Germany) filled with 3 M KCl. The resistance of the electrode ranged from 50 to 80 M\(\Omega\). Electrical responses of smooth muscle cell were recorded by penetrating the electrode from the mucosal side. The transmembrane potentials thus recorded were displayed on a cathode ray oscilloscope (SS-7602, Iwatsu, Tokyo, Japan) through a preamplifier (MEZ-8301, Nihon Kohden, Tokyo, Japan). The data were also acquired and input into a personal computer (Dell Computer Inc., Kawasaki, Japan) through an A/D converter (Axon Instruments Inc., CA, USA) at 500 Hz, filtered at 100 Hz, and analyzed with Axoscope 7 (Axon Instruments Inc.).

The ionic composition of the Krebs solution used was as follows (in mM): Na\(^{+}\) 137.4, K\(^{+}\) 5.9, Ca\(^{2+}\) 2.5, Mg\(^{2+}\) 1.2, Cl\(^{-}\) 134, HCO\(_3^{-}\) 15.5, H\(_2\)PO\(_4^{-}\) 1.2, and glucose 11.5. The solution was aerated with O\(_2\) containing 5% CO\(_2\), and the pH of the solution was maintained at 7.2 to 7.4. Ca-free solution was prepared by omitting CaCl\(_2\) from the Krebs solution.

The drugs used were as follows: prostaglandin F\(_{2a}\) (Ono Pharmaceutical Co., Osaka, Japan), nifedipine, niflumic acid, 4,4'-disothiocyanostilbene-2,2'-disulfonic acid (DIDS), cyclopiazonic acid (CPA), caffeine and 1,2-bis(2-aminoophenoxy)ethane-N,N,N',N'-tetraacetic acid (as its acetoxymethyl ester, BAPTA-AM), indomethacin, and tetrodotoxin (all from Sigma, St. Louis, MO, USA). Nifedipine and niflumic acid were dissolved in ethanol at concentrations of 10 and 100 mM, respectively. BAPTA-AM was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 5 mM. Indomethacin was dissolved in 5 mM Na\(_2\)CO\(_3\) solution at a concentration of 5 mM. The remainders were dissolved in distilled water at concentrations of 1 mM. These drugs were kept in a refrigerator and diluted further with the Krebs solution just before use to obtain the desired concentrations. The solvents themselves did not alter the pH of the Krebs solution.

The data were expressed by the mean ± standard deviation (SD). The statistical comparison was made by using a Student’s t-test, and probabilities of less than 5% (\(p<0.05\)) were considered significant.
RESULTS

Spontaneous activities of the smooth muscle cells in the guinea-pig renal pelvis

All smooth muscle cells examined in the proximal pelvic region generated spontaneous electrical activities. About 10% of the cells (n=14) generated triangle-formed potentials with slow rising and falling phases (Fig. 1A), and the mean value of the rate of rise of the potential was 65.8±24.6 V s⁻¹ (n=14). These potentials appeared at 4.4±1.3 times min⁻¹, and their peak amplitude and duration were 16.5±6.8 mV (n=14) and 1.1±0.8 s (n=14), respectively. In these cells, the membrane potential at the most negative level was −39.4±6.7 mV (n=14). These slow oscillatory potentials resembled the pacemaker potential recorded in the renal pelvis [6, 8]. Most cells examined (n=105) showed a rhythmic generation of plateau-type potentials with initial spike components (Fig. 1B), and they were considered the conducting-type potential [6, 8]. The amplitudes of the initial spike potential and plateau component were 36.4±9.9 and 26.6±6.8 mV, respectively (n=105 for each). The rate of rise of the spike activity was 308.8±162.1 V s⁻¹ (n=51), and the duration of the plateau component was 1.3±0.7 s (n=51). A repetitive generation of spike potentials with decreasing amplitude was often observed during the initial phase of the potential. The membrane potential of cells generating the conducting-type potential measured at the most negative state was −49.3±8.2 mV (n=105).

The frequency of generating the conducting type potentials was 3.4±1.2 times min⁻¹. These potentials were not modulated by 0.5 μM tetrodotoxin (data not shown), suggesting that they were myogenic activity. The experiments were carried out to investigate mainly the properties of the conducting-type potentials, and properties of the pacemaker potentials were carried out only partially, since most cells generated only the conducting-type potentials. All tissues examined were active with the generation of these potentials for about 5 h, then ceased generating any spontaneous activity.

Effects of inhibitors of Ca²⁺ and Cl⁻ channels on spontaneous activity

In Ca²⁺-free solution, smooth muscle of the renal pelvis stopped generating the spontaneous generation of conducting-type potentials, with no detectable changes in the membrane potential (control, −48.3±2.8 mV, n=4; Ca²⁺-free solution, −47.4±3.4 mV, n=4; p>0.05). In the presence of 1 μM nifedipine, the spontaneous activities, both pacemaker and conducting-type potentials, disappeared. In cells producing conducting-type potentials, the membrane was depolarized by nifedipine (control, −46.8±3.4 mV, n=4; in nifedipine, −43.5±3.4 mV, n=4, p<0.05, Fig. 2). The inhibition of conducting-type potentials appeared initially in the reduction in amplitude of the initial spike potentials and duration of the plateau component (Fig. 2B). These observations indicate that an influx of Ca²⁺ through voltage-gated L-type Ca²⁺ channels is essential for the generation of spontaneous activity in pelvic smooth muscle cells.

A possible involvement of Ca²⁺-activated Cl⁻ channels in the generation of spontaneous activity was determined by applying niflumic acid (0.1 mM) and DIDS (0.1 mM), both being an inhibitor of Ca²⁺-activated Cl⁻ channels [17–20]. Niflumic acid inhibited the generation of spontaneous activity with no obvious depolarization of the membrane (control, −51.5±
5.9 mV, n=4; in niflumic acid, −50.3±6.0 mV, n=4; p>0.05; Fig. 3A). The inhibition of spontaneous activity by niflumic acid developed slowly with reduced frequency before terminating the activity. The expanded record of the activity shows that the inhibition appears first on the plateau potential, which causes a repetitive generation of spike potentials (Fig. 3B). An application of ethanol (1%), the solvent of niflumic acid, showed no effect on the membrane potential and spontaneous activity (n=3, data not shown). DIDS did not modulate the spontaneous activity or membrane potential in smooth muscle of the renal pelvis (n=5; Fig. 3C and D).

The role of intracellular Ca\(^{2+}\) on the spontaneous activity

Attempts were made to modulate intracellular Ca\(^{2+}\) concentrations or internal Ca\(^{2+}\) store to reveal the Ca\(^{2+}\)-dependent properties of spontaneous activity in smooth muscle of the renal pelvis. Cyclopiazonic acid (CPA) was used to inhibit Ca\(^{2+}\)-ATPase at the internal Ca\(^{2+}\) store [16], and BAPTA was used to chelate intracellular Ca\(^{2+}\) [13]. A depletion of Ca\(^{2+}\) from the internal store was also elicited by caffeine [14, 15].

CPA (10 μM) showed a dual effect on the membrane electrical activities: an initial excitation with a subsequent inhibition of spontaneous activity and successive depolarization of the membrane (Fig. 4A). The initial excitation appeared 3 to 6 min after CPA was applied, with an increase in the frequency of spontaneous activity. Within 10 to 15 min, the spontaneous activity successively reduced in frequency and finally disappeared. During the inhibitory processes, the duration of the spontaneous activity was doubled (control, 1.6±0.6 s; in 10–15 min CPA, 3.1±1.7 s, n=4) (Fig. 4B). The depolarization of the membrane reached the stable level of −40.0±5.7 mV (n=4) in 10 to 13 min after superfusion with CPA-containing solution, and the value was significant from the resting potential −43.0±7.1 mV (n=4, p<0.05). These effects of CPA were reversible, and 30–60 min were required for the recovery (data not shown).

Caffeine (1 mM) instantaneously inhibited the spontaneous activity with an associated depolarization of the membrane (control, −43.3±4.2, n=4; in caffeine, −39.5±4.7 mV, n=4; p<0.05). The recovery from the caffeine-induced inhibition was very rapid, and the activity reverted to the control level within 3 min (Fig. 4C).

Application of BAPTA-AM (30 μM) increased the frequency of spontaneous activity (control, 2.9±1.3 times min\(^{-1}\), n=4; in BAPTA for 10 to 15 min, 3.7±1.5 times min\(^{-1}\), n=4, p<0.05), with no significant alteration of the membrane potential (control, −42.0±4.1 mV, n=4; in BAPTA for 10 to 15 min, −43.0±3.5 mV, n=4; p>0.05). In the presence of BAPTA, the amplitude of spontaneous activity increased and finally reached a stable amplitude of more than 1.5 times the control in about 10 min (control, 27.0±2.0 mV, n=4; in BAPTA, 47.0±12.0 mV, n=4;
Modulation of the spontaneous activity by prostaglandins

The effects of inhibition of an enzyme cyclooxygenase by indomethacin on spontaneous activity were observed in smooth muscle of the renal pelvis. Indomethacin (5 μM) inhibited the generation of spontaneous activity (data not shown), with no alteration of the membrane potential (control, −48.5 ± 2.1 mV, n = 5; in indomethacin, −48.0 ± 2.5 mV, n = 5; p < 0.05), the results being confirmed in the previous observation [9]. In smooth muscle of the renal pelvis, prostaglandin F2α (PGF2α, 600 nM) increased the frequency of spontaneous activity (control, 2.0 ± 0.9 times min⁻¹, n = 3; in PGF2α, 3.4 ± 1.4 times min⁻¹, n = 3; p < 0.05), with no alteration of the membrane potential (control, −42.0 ± 6.2 mV, n = 3; in PGF2α, −40.0 ± 5.1 mV, n = 3; p > 0.05; Fig. 6A). In the presence of PGF2α, the duration of the plateau component was increased (control, 1.5 ± 0.2 s, n = 3; in PGF2α, 1.7 ± 0.2 s, n = 3; p < 0.05), with no significant change in the amplitude of spike and plateau potentials (Fig. 6B). The excitatory actions of PGF2α did not appear in the presence of 1 μM nifedipine (n = 3, data not shown).

The spontaneous activity of pelvic smooth muscles ceased about 5 h after preparing the tissues, with no significant alteration of the resting potential (active cells; −42.1 ± 3.1 mV, n = 7; quiescent cells, −42.8 ± 3.9 mV, n = 7; p > 0.05). However, the application of PGF2α could elicit a generation of spontaneous activity again in quiescent cells (Fig. 6C). The forms of the evoked activity were similar to those generated spontaneously.

DISCUSSION

The cellular mechanisms for the generation of spontaneous activity in smooth muscle remain unclear. In lymphatic [21] and urethral smooth muscles [22], the spontaneous activity is inhibited by niflumic acid, cyclopiazonic acid, or BAPTA, indicating that the activity is initiated by opening Ca²⁺-activated Cl⁻ channels activated through the release of Ca²⁺ from internal stores. In gastrointestinal tissues, the pacemaker of spontaneous activity may be interstitial cells [23, 24]. The spontaneous activity appearing in the interstitial cells is initiated by depolarizing the membrane through “low-threshold” Ca²⁺ channels [25] or Ca²⁺-activated Cl⁻ channels activated by Ca²⁺ released from the internal stores [26]. The myogenic activities of gastric smooth muscles isolated from the guinea-pig antrum disappear in the presence of cyclopiazonic acid or BAPTA, but not in the presence of niflumic acid [27]. The possible involvement of spontaneous production of inositol 1,4,5-trisphosphate (InsP₃), an activator of the Ca²⁺ channel in the internal Ca²⁺ stores [15], is also suggested [28]. Thus the cellular mechanisms for generation of spontaneous activity in
smooth muscle are equivocal.

The present experiments indicated that in smooth muscle of the guinea-pig renal pelvis, the spontaneous electrical activity stopped with nifedipine, caffeine, or CPA. Caffeine and CPA have actions to deplete internal Ca\(^{2+}\) stores by different mechanisms [14–16]. Thus the results suggest that Ca\(^{2+}\) release from the internal store is essential for a generation of the activity, as with smooth muscles of the lymphatic vessel [21] or urethra [22]. The important difference, however, appears on the effectiveness of nifedipine on the spontaneous activity. The transient depolarization probably triggered by Ca\(^{2+}\) released from the internal stores is not inhibited by nifedipine in lymphatic and urethral smooth muscles [21, 22]. In the renal pelvis, the spontaneous activity is inhibited by nifedipine [9], and this is also confirmed in the present experiments. Nifedipine is an inhibitor of the voltage-gated L-type Ca\(^{2+}\) channels, indicating that the pelvic activity is initiated by an influx of Ca\(^{2+}\) from the external media. It is speculated that Ca\(^{2+}\) entering the cell activates Ca\(^{2+}\)-induced a Ca\(^{2+}\) release mechanism [14] in the internal store.

Ionic mechanisms for generating spontaneous activity in smooth muscles are also equivocal. In cultured interstitial cells [26] and lymphatic [21] and urethral smooth muscles [22], the spontaneous membrane depolarization is inhibited by niﬂumic acid. A reasonable explanation is that the elevated intracellular Ca\(^{2+}\) stimulates Ca\(^{2+}\)-activated Cl\(^{-}\) channels and depolarizes the membrane. This seems also to be so in the renal pelvis of the guinea-pig, and the spontaneous activity is inhibited by niﬂumic acid. However, the activity is not inhibited by DIDS, another inhibitor of the Ca\(^{2+}\)-activated Cl\(^{-}\) channels. The inhibitory actions of both niﬂumic acid and DIDS on the Ca\(^{2+}\)-activated Cl\(^{-}\) channels are found in vascular smooth muscles, but it may not be so in pelvic smooth muscles. Alternatively, the effects of niﬂumic acid on the pelvic activity are not related to the inhibition of Cl\(^{-}\) channels. Thus the present experiments could not confirm a possible involvement of Ca\(^{2+}\)-activated Cl\(^{-}\) channels in the generation of spontaneous activity in smooth muscle of the guinea-pig renal pelvis.

Spontaneous electrical activity of smooth muscles in the guinea-pig renal pelvis is inhibited by indomethacin, thereby suggesting that endogenous prostaglandins are responsible for initiation of the activity [9, 10]. The present experiments also confirmed that indomethacin inhibits spontaneous activity, furthermore, the supporting evidence was the excitation of pelvic smooth muscle cells by PGF\(_{2\alpha}\). The excitatory actions of PGF\(_{2\alpha}\) appear only in the absence of nifedipine, indicating that PGF\(_{2\alpha}\) accelerates Ca\(^{2+}\) influx through the voltage-gated Ca\(^{2+}\) channels and initiates membrane activities. The PGF\(_{2\alpha}\) could recover the spontaneous action potentials with regular rhythm, even in the quiescent cells.

BAPTA incorporated in the cell chelates intracellular Ca\(^{2+}\), thus preventing the Ca\(^{2+}\)-dependent responses [13], and this may also be the same in some spontaneously active smooth muscle cells. In particular, the spontaneous activities appearing in lymphatic [21], urethral [22] and gastric smooth muscles [27] are inhibited by BAPTA. The spontaneous activity of smooth muscle in the guinea-pig renal pelvis, however, is not inhibited by BAPTA; instead, the amplitude of spike potentials is increased. The spike potential may be produced by an influx of Ca\(^{2+}\), since this potential is sensitive to nifedipine. It is speculated that in smooth muscle of the renal pelvis, BAPTA may be insufficient to chelate intracellular Ca\(^{2+}\) below threshold to prevent the activity of Ca\(^{2+}\)-dependent mechanisms. As a consequence, the amplitude of spike potential may be increased in the presence of BAPTA because of an increased gradient of Ca\(^{2+}\) concentrations across the membrane.

In summary, the present experiments indicate that the spontaneous activity appearing in smooth muscle of the renal pelvis has causal relation to the release of Ca\(^{2+}\) from the internal stores, and the influx of Ca\(^{2+}\) through voltage-gated Ca\(^{2+}\) channels may be an essential factor to drive the mechanism. A contribution of Ca\(^{2+}\)-activated Cl\(^{-}\) channels for generation of the activity is not confirmed. Endogenous prostaglandins may be important as an initiator of the spontaneous activity in pelvic smooth muscles.

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