Gap junction function in vascular smooth muscle: influence of serotonin

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Moore, Lisa K., and Janis M. Burt. Gap junction function in vascular smooth muscle: influence of serotonin. *Am. J. Physiol.* 269 (*Heart Circ. Physiol.* 38): H1481–H1489, 1995.—In this study we examined the effects of serotonin (5-hydroxytryptamine, 5-HT) on the function of gap junctions between smooth muscle cells isolated from human and pig coronary and rat mesentery arteries and between A7r5 cells (cell line derived from embryonic rat aorta). Mesentery and pig coronary cells expressed connexin (Cx) 43, and human coronary cells expressed Cx40. Mesentery and pig coronary cells each exhibited a single gap junction channel population with unitary conductances of 75 and 59 pS, respectively. Human coronary cells exhibited two channel populations with unitary conductances of 51 and 107 pS. The A7r5 cells express Cx40 and Cx43 and exhibit three channel populations with unitary conductances of 70, 108, and 141 pS. Under control conditions, junctional conductance between the four cell types ranged from 11 to 20 nS. During maximal stimulation with 5-HT (1–10 μM), junctional conductance increased (29–75%) in all four cell types. The unitary conductance profiles in the rat mesentery and pig coronary cells were unaffected by 5-HT, suggesting that the observed increase in macroscopic conductance reflects an increase in open probability. Unitary conductances were also unaffected in the human coronary and A7r5 cells. However, there was a reduced frequency of the 105-pS channel in the human coronary cells and of the 70- and 141-pS channels in the A7r5 cells. These changes in the relative frequency histograms suggest that the open probabilities of the various channel types are differentially affected by the 5-HT treatment. Thus the data suggest that vascular smooth muscle cells exhibit a variety of channel types whose open probabilities are differentially affected by 5-HT stimulation.

connexin40; connexin43; coronary smooth muscle; mesenteric vascular smooth muscle; A7r5 cells; intercellular communication; 5-hydroxytryptamine

FUNCTIONAL GAP JUNCTIONS are necessary for coordinated regulation of tone and flow in the vasculature (7, 32–34). Vessel diameter is modulated by serotonin (5-hydroxytryptamine, 5-HT); however, the magnitude and direction of diameter change are vessel specific and dose and pathophysiological state dependent (4, 8, 12, 27, 36). The mechanistic basis for these differences in vessel responsiveness remains unknown. Although other receptor types have been described, the principle 5-HT receptor operative in vascular smooth muscle (VSM) is the 5-HT_2 subtype, whose effects are mediated via G protein activation of phospholipase C and subsequent release of inositol trisphosphate (which mobilizes intracellular calcium) and diacylglycerol (which activates protein kinase C) (17).

Gap junctions are aggregates of channels that span the membranes of neighboring cells and mediate the movement of ions and small molecules between cells. Each channel is composed of two hexamers of proteins termed connexins, one hexamer from each cell, that join in the extracellular space to form the patent channel. Multiple connexins have been identified. They share significant homology in the membrane spanning and extracellular domains but differ considerably in their cytoplasmic domains (13, 44) and consequently functional regulation. Of the many members of the connexin gene family, only connexins (Cx) 43 and 40 have been found in VSM (1, 2, 18, 21, 24, 25); the extent of their expression appears to vary among vessels. Channels of these two connexin types exhibit different unitary conductances and permeability characteristics (40); hybrids of these two connexins (connexon of Cx40 pairing with connexon of Cx43) do not to form in the oocyte expression system (3) and may not form in mammalian cells (25).

Gap junction function and expression are modulated by second messenger systems. The function of Cx43 channels is modulated by second messenger systems that result in phosphorylation of the channel proteins (19, 20, 28–30, 39, 40). Cx40 is also a phosphoprotein (40), raising the possibility that its function may also be modulated by second messenger systems. Differences in channel function, permeability, and regulation and the variation in expression levels of Cx40 and Cx43 among vessel beds could combine to provide varied vessel response to 5-HT.

In this study we explored this possibility by examining connexin expression and gap junction function as modulated by 5-HT in several VSM cell types. We demonstrate that smooth muscle cells (SMCs) from coronary, mesentery, and aorta (A7r5 cells) differed in their expression of connexins and channel function. Despite such differences the response of all cell types to short-term (<20 min) exposure to 5-HT was increased electrical coupling. However, the mechanisms underlying increased coupling differed between the cell types.

MATERIALS AND METHODS

Cell culture. A7r5 cells, a SMC line originating from neonatal rat thoracic aorta, were obtained from American Type Culture Collection at *passage 12* and maintained in culture with Dulbecco's modified Eagle's medium (DMEM; GIBCO) supplemented with 10% fetal calf serum and 0.1% antibiotic solution. Cells were maintained in a humidified 5% CO_2 incubator at 37°C. Cells were passaged weekly with 0.25% trypsin in Ca^{2+}-Mg^{2+}-free phosphate-buffered saline (PBS) (24). Cells were not used after *passage 20*.

Human coronary vessels were obtained from recipient heart tissue following transplant surgery. Tissue was received within 1 h and was maintained in cold sterile saline until time of culture. Primary cultures of pig and human coronary cells were obtained using an explant method (38). Vessels (left anterior descending or circumflex arteries) were manually stripped of adventitia, and the endothelial cell layer was

removed by gently scraping the intimal lining with a scalpel blade. The remaining smooth muscle layer was then minced into 1-mm^2 pieces and placed in 60-mm culture dishes precoated with 1% gelatin solution in PBS. Small glass coverslips were used to "weight" the tissue down so that it remained in contact with the dish. A small amount of DMEM was added to cover the tissue, and the dishes were placed in the incubator. After 4–5 days cells could be observed growing out from the explants. Cells were confluent after 3–4 wk and passaged with the same methodology as used for the A7r5 cells. Most of our studies were done on cells from *passages 3–6*; however, no major changes in connexin function or expression were noted in *passages 3–8*. Cells were not used after *passage 8*.

For isolation of mesentery SMCs, Wistar-Kyoto rats were anesthetized with ether. The abdomen was prepared with alcohol, and a midline incision was made through the skin and the peritoneal lining. The mesentery was quickly dissected away from loops of intestine and placed in sterile PBS for further dissection. The mesenteric artery arcades were dissected free of extraneous tissue and placed in a dissociation mixture consisting of a balanced salt solution with 0.2 mM Ca^{2+}, 15 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer, 0.125 mg/ml elastase (Pancreopeptidase, type III, 90 U/mg, Sigma Chemical, St. Louis, MO), 1.0 mg/ml collagenase (CLS type I, 135–180 U/mg, Worthington Biochemical, Freehold, NJ), and 2.0 mg/ml bovine albumin (Sigma) as described by Gunther et al. (14). This solution was filter sterilized before use. Tissue dissociation was carried out at 37°C in a shaker bath for up to 1 h. The tissue was then triturated and strained through sterile 100-μm mesh screen, and the resulting cells were placed in 60-mm culture dishes with DMEM containing 10% fetal calf serum. Cells were maintained in a humidified environment in 5% CO_2 in air at 37°C. Cells were passaged as described above, and *passages 3–6* were used in our studies. No major changes in connexin expression or function were observed over these passages.

Smooth muscle character of all cells was confirmed by staining with smooth muscle-specific α-actin monoclonal antibody (data not shown). Absence of endothelial cell contamination was verified by staining for von Willebrand factor, an endothelial cell-specific marker.

Electrophysiology. For dual whole cell voltage-clamp experiments, cells were trypsin digested and plated at low density on 25-mm coverslips. A7r5 cells typically attached to the coverslips and began to flatten quickly; they were ideally suited for patch clamping between 1 and 4 h postplating. The human and pig coronary cells were not well attached to the coverslips until 3 h and were most easily patched up to 8 h postplating. The mesentery SMCs were intermediate between the A7r5 and the coronary cells. Once cells were adequately attached, coverslips were placed in an experimental chamber and perfused at 5 ml/min with either 70 μM or 2 mM Ca^{2+} salt solution containing (in mM) 130 NaCl, 4 KCl, 1 $MgCl_2$, 5 HEPES, 5 dextrose, 2 pyruvate, 1 $BaCl_2$, and 2 CsCl (31). 5-HT or ketanserin (from stock solutions) was brought to final concentration with one of these solutions and perfused over cells at the same rate.

Dual whole cell voltage clamp was used to determine macroscopic junctional and single-channel conductance. Macroscopic conductance was determined by voltage clamping both cells of a cell pair to a 0-mV holding potential. Command potential pulses of −10 mV were alternately applied to each cell, and junctional current was measured in the opposite cell. Junctional conductance (G_j) was determined by dividing the junctional current by the transjunctional voltage difference. This same general methodology was used to determine unitary channel conductance. In cell pairs having a naturally low G_j or in pairs reversibly uncoupled with 2–4 mM halothane (which has previously been demonstrated to have no effect on unitary conductance relative frequency profiles; Refs. 5, 24, 35), a 20- to 70-mV (most often 40–50 mV) transjunctional driving force was applied. Gap junction channel events (openings and closings) could be recognized by their equal amplitude but opposite polarity in the two current traces. Unitary conductances were determined by dividing the amplitudes (measured by caliper from chart recording with an accuracy of ±1.25 pS) of opening or closing events (in pA) by the transjunctional voltage. Data were digitized on Neurocorder model DR 484 for storage on a videotape and displayed directly on a Gould Brush recorder. Filtering ranged from 10 to 1,000 Hz depending on the cell pair. For each cell pair, events were binned (10 pS) and the frequency of events in each bin relative to events in all bins was calculated. The mean relative frequency for each bin from all cell pairs in a treatment group was then calculated and plotted as a histogram. The data in these frequency histograms were fit (Peakfit, Jandel Scientific) with from one to four Gaussian peaks. The fit with the best F value (and r^2 value) in which all peak center parameter values, which represent unitary conductance of the channel, were significant was considered to represent the best fit. Unitary conductances are expressed as means ± SD throughout.

Patch-type microelectrodes having resistances of 3–7 MΩ were pulled from 1.2-mm-diameter glass (AM systems) and filled with a patch solution containing (in mM) 67 potassium glutamate, 67 CsCl, 10 tetraethylammonium chloride, 0.5 $CaCl_2$, 0.3 $MgCl_2$, 10 ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, 10 KCl, 10 HEPES, 5 glucose, and 5 K_2ATP.

Northern blot analysis. Total RNA was isolated from three or four 100-mm dishes of confluent cells. Cells were washed with PBS and placed in guanidine isothiocyanate. RNA was obtained following phenol-chloroform extraction (6). RNA (10 or 15 μg) was electrophoresed on 1% agarose-formaldehyde gels followed by capillary blotting onto nylon membranes (Hybond N, Amersham). RNA was then cross-linked to the membrane by exposure to 300-nm ultraviolet transillumination for 3–5 min. cDNA probes to rat Cx43 (bases 1–1390) and Cx40 (bases 1–591) (both generously provided by E. C. Beyer) and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH; bases 1–770) were prepared by isolation of the DNA fragments from low-melting-point agarose gels and radioactively labeled with [^{32}P]dCTP using the Klenow fragment of DNA as described previously (24) and modified from Feinberg and Vogelstein (11). Membranes were prehybridized for 30 min at 65°C before overnight hybridization at the same temperature in a solution containing 0.75 M Na_2HPO_4, 1% sodium dodecyl sulfate, and 0.1% nonfat dry milk as a blocker for nonspecific binding as well as the labeled GAPDH and either Cx43 or Cx40 probes. High-stringency washes were performed as described by Moore et al. (24), followed by exposure to Kodak XAR film at −80°C.

RESULTS

Connexin expression. Rat mesentery, human and pig coronary, and A7r5 SMCs were screened for expression of Cx40 and Cx43, the only connexins that have previously been found in VSM cells (Fig. 1). Cx43 mRNA was detected in the rat mesentery, pig coronary, and A7r5 cells. Cx40 mRNA was detected in the human coronary and A7r5 cells. Although expresion of Cx43 in the human coronary cells and Cx40 in the mesentery and pig coronary cells cannot be ruled out, these data suggest that, if present, the levels of these mRNAs must

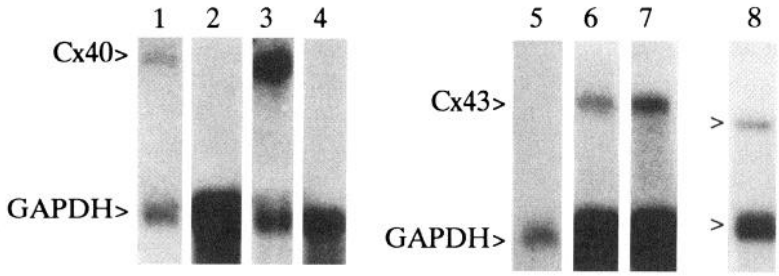

Fig. 1. Expression of connexins (Cx) 40 and 43 mRNAs in vascular smooth muscle (VSM) cells. Cx40 was found in human coronary VSM and A7r5 cells (*lanes 1* and *3*) but not pig coronary and rat mesentery VSM cells (*lanes 2* and *4*). Cx43 was found in pig coronary VSM, A7r5, and rat mesentery VSM cells (*lanes 6–8*) but not human coronary VSM cells (*lane 5*). GADPH, glyceraldehyde-3-phosphate dehydrogenase.

be significantly less than the levels of the detected mRNAs.

Electrophysiology. The potential effect of 5-HT on junctional conductance was evaluated in each cell type using dual whole cell voltage clamp. Ketanserin was used to assess involvement of the 5-HT_2-receptor subtype in observed responses. Each cell of a pair was whole cell clamped under control conditions. After stability of coupling was evident, 1 or 10 μM 5-HT was introduced into the perfusion chamber. These concentrations have previously been shown to produce half-maximal or maximal stimulation of inositol monophosphate production in A7r5 cells (10). An increase in junctional current was observed within 1 min of application of drug in all cell types, consistent with the time course of inositol monophosphate production (10). Response was maximal (25–80% increase, independent of drug concentration) by 2–3 min and was typically sustained for the duration of the recording (Fig. 2*A*; see Table 1 for summary of data). In some cell pairs the increase in junctional conductance was transient, with conductance returning to pretreatment values after 4–5 min of exposure (Fig. 2*B*). In a similarly designed set of experiments, we found that 0.1 and 1 μM ketanserin had no effect on junctional conductance (Table 1). Pretreatment of cells with ketanserin prevented subsequent response of the cells to 5-HT even when 5-HT was present at 10- to 100-fold higher concentrations than the antagonist (Table 1).

Junctional conductance is the sum for each channel type of the product of unitary conductance (γ), open time probability (P_o), and number of channels in the junction. Consequently, an increase in G_j could reflect an increase in any one of these parameters for any of the expressed channel types or a simultaneous decrease in one parameter offset by an increase in another. Thus, although 5-HT caused an increase in G_j in all cell types, the underlying mechanisms could differ.

To evaluate possible differences in mechanism of 5-HT effect, the unitary conductances of expressed channels in each cell type were evaluated under control and treatment conditions using dual whole cell voltage clamp. Pairs of cells were whole cell clamped under control conditions or after short-term exposure to 5-HT. When pairs were too well coupled to allow resolution of single-channel events, halothane was used to reduce coupling. As in previous studies (5, 24, 35), no obvious differences in unitary conductance relative frequency profiles were discernible between records from cell pairs exposed or not to halothane. Analysis of single-channel events from pig coronary SMCs revealed the presence of a single-channel population with a unitary conductance of 59 ± 9.8 pS (Fig. 3*A*). Similarly, the rat mesentery SMCs exhibited a single population of channels with a unitary conductance of 75 ± 9.8 pS (Fig. 4*A*). The human coronary SMCs exhibited two channel populations with unitary conductances of 51 ± 8.5 and 107 ± 26 pS (Fig. 5*A*). The 51-pS channel constituted 36% of the total number of events. The A7r5 cells exhibited three channel populations with unitary conductances of 70, 108 and 141 pS (25). Each of these channel types constituted one-third of the total population. These data indicate species, vascular bed or Cx-specific regulation of gap junction channel unitary conductance. Connexin expression and function for these cell types are summarized in Table 2.

To determine whether the increase in macroscopic conductance produced by 5-HT involved a change in the unitary conductances of expressed channels, we evaluated single-channel events after short-term (<20 min)

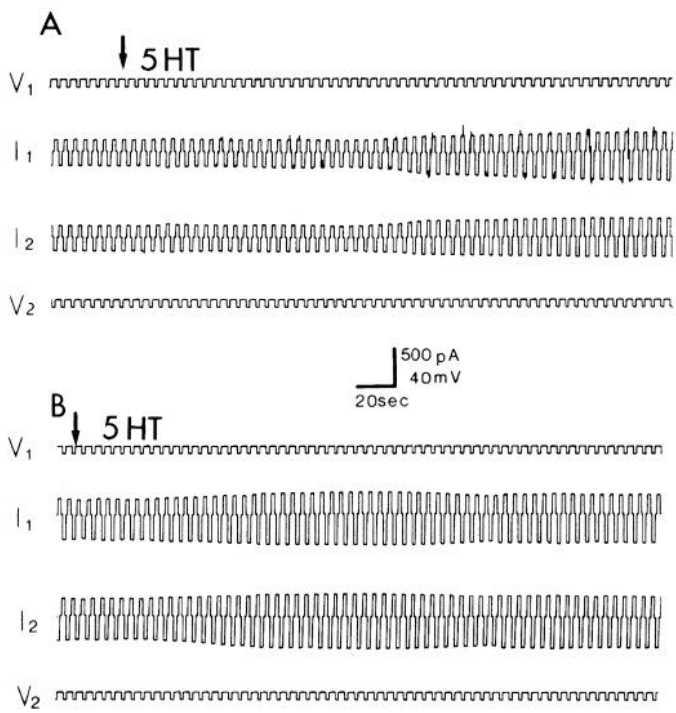

Fig. 2. Influence of 1 μM serotonin (5-HT) on junctional conductance. 5-HT typically produced a sustained increase in junctional conductance that was maximal within 2–3 min; however, in some cell pairs increase was transient. *A*: examples of a sustained 43% increase observed in a pair of A7r5 cells. *B*: a transient 42% increase observed in a pair of pig coronary cells. In both cases, cells were clamped at 0 mV and stepped alternately to −10 mV. V_1 and V_2, voltage in *cells 1* and *2*, respectively; I_1 and I_2, junctional (upward deflection) and total current (downward deflection) for *cells 1* and *2*, respectively.

Table 1. *Junctional conductance for various SMC types under control conditions and increase in junctional conductance during various treatments*

Treatment	Mesentery	Coronary (Pig)	Coronary (Human)	A7r5
		Junctional conductance, nS		
Control	11.8 ± 0.3 (12)	20.5 ± 0.1 (22)	14 ± 3.3 (11)	11 ± 2.1 (31)
		%Increase in junctional conductance		
5-HT (1–10 μM)	+29 ± 11 (4)	+44 ± 9 (8)	+26 ± 8 (8)	+79 ± 29 (8)
Ketanserin	ND	−3 ± 4.3 (3)	+1 ± 7 (4)	3 ± 2.7 (3)
Ketanserin and 5-HT	ND	−8.6 ± 5.4 (3)	+1.3 ± 1.3 (3)	ND

Values are means ± SE for no. of cells given in parentheses. ND, not determined; SMC, smooth muscle cell; 5-HT, serotonin.

exposure to 5-HT (data summarized in Table 2). As illustrated in Fig. 6, *A* and *B*, both the pig coronary and rat mesentery SMCs continued to exhibit predominantly one channel size. Analysis of many of these events from multiple cell pairs confirmed the presence of a single population of channels in both cell types (Figs. 3*B* and 4*B*). In contrast, both the human coronary SMCs and A7r5 cells exhibited striking changes in the relative frequencies of their channel populations after exposure to 5-HT. In the human coronary SMCs, channels of multiple sizes were observed following 5-HT (Fig. 7). However, analysis of these events from multiple cell pairs revealed that the 51-pS channel, which constituted only ~36% of the total channel population under control conditions, constituted >90% of the population after 5-HT treatment (Fig. 5*B*). The frequency of the remaining channels was too low to be fit as a distinct peak. Similarly, in the A7r5 cells the relative frequencies of the 70- and 141-pS channels, each of which constituted approximately one-third of the total population under control conditions, were diminished during 5-HT exposure, and the frequency of the 108-pS channel was enhanced (Figs. 8 and 9).

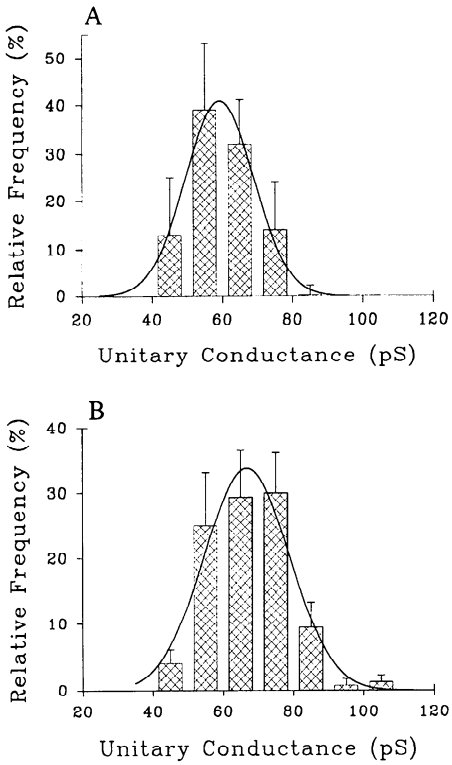

Fig. 3. Relative frequency of events-amplitude histograms of single-channel events in pig coronary VSM cells under control conditions (*A*) and during 5-HT treatment (*B*). Cells exhibited a single-channel population with a mean unitary conductance of 59 ± 9.8 (SD) pS under control conditions. Data were from 4 cell pairs, 422 events (F = 237, r^2 = 0.987). Cells exposed for several minutes to 1 μM 5-HT also exhibited a single population of channels with a unitary conductance of 67 ± 12 pS. Data were from 6 cell pairs, 513 events (F = 71, r^2 = 0.959). Error bars, SE.

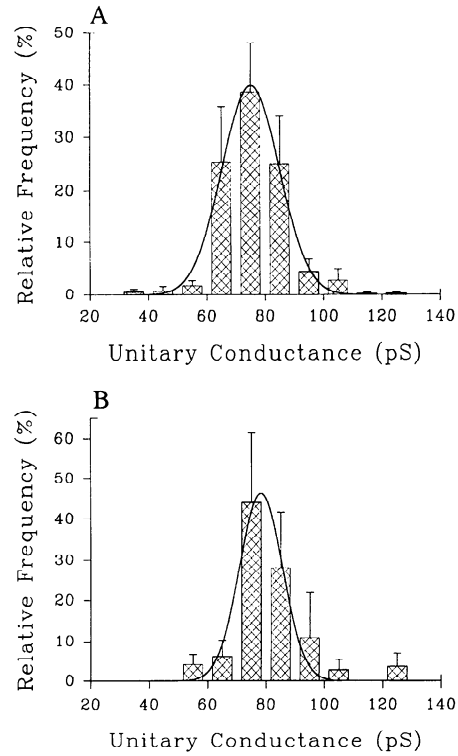

Fig. 4. Relative frequency of events-amplitude histograms of single-channel events observed in rat mesentery VSM cells under control conditions (*A*) and during 5-HT treatment (*B*). Cells exhibited a single population of channels with a mean unitary conductance of 75 ± 9.8 pS under control conditions. Data were from 8 cell pairs, 918 events (F = 275, r^2 = 0.987). Cells exposed for several minutes to 1 μM 5-HT also exhibited a single population of channels with a mean unitary conductance of 78 ± 7.5 pS. Data were from 4 cell pairs, 182 events (F = 61, r^2 = 0.946). Error bars, SE.

DISCUSSION

In this study we sought to determine whether the gap junctions between SMCs isolated from different vessels and species exhibited comparable responsiveness to 5-HT. We found that connexin expression and channel function varied in SMCs isolated from vessels of different species or organs. Despite these differences all of the examined SMC types exhibited increased coupling on exposure to 5-HT. This response was blocked by ketanserin, indicating a 5-HT_2-receptor-mediated response.

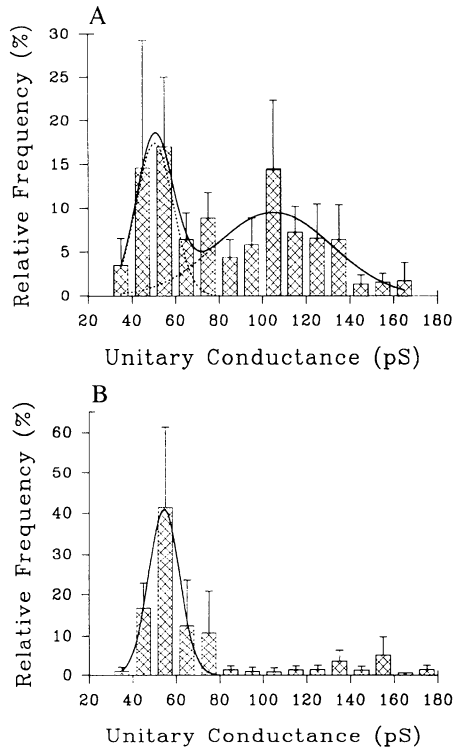

Fig. 5. Relative frequency of events-amplitude histogram of single-channel events observed between human coronary VSM cells under control conditions (*A*) and during 5-HT treatment (*B*). Under control conditions, 2 channel populations with mean conductances of 51 ± 8.5 and 107 ± 26 pS were observed. Data were from 5 cell pairs, 909 events (F = 6.6, r^2 = 0.807). Peak (51 pS) constituted 36% of total channel population. Although channel events of multiple sizes were observed during exposure to 5-HT, data were best fit by only 1 peak with a unitary conductance of 54 ± 7.2 pS. Data from 3 cell pairs, 659 events (F = 66, r^2 = 0.911). Error bars, SE.

Although coupling was increased in all cell types, the underlying mechanisms at the channel level differed. Thus variation in connexin expression, function, and mechanism of response to common stimuli could contribute to observed differences in physiological responsiveness of blood vessels from different organs and species.

The connexins are a gene family consisting of at least 12 members. Of these only two have been found in VSM, Cx43 and Cx40 (1, 2, 18, 21, 24, 25). Controversy continues over potential differences in expression of Cx40 and Cx43 in intact vessels vs. isolated cell preparations thereof and between vascular beds. Cx43, but not Cx40, has been localized to the smooth muscle layer of large vessels. However, Cx40 and Cx43 are both clearly present in the smooth muscle layer of at least some resistance vessels (21). The results presented herein indicate that Cx40 is expressed by diseased human coronary SMCs. Interestingly, these cells did not contain detectable Cx43 despite its presence (and Cx40's apparent absence) in the pig coronary SMCs. Thus our data suggest that cultured vascular SMCs from different species, vessel beds, and developmental ages express different connexins.

Table 2. *Connexin expression and function in SMCs*

SMC Source	Cx mRNA Expressed	Unitary Conductance, pS	
		Control	5-HT
Rat mesentery	Cx43	75	78
Pig coronary	Cx43	59	67
Human coronary	Cx40	51, 107	54
A7r5 cells (rat aorta)	Cx43,Cx40	70, 108, 141	99

Cx, connexin.

In addition to expression of different connexins, our data indicate that the functional channels formed in these four cell types exhibit different unitary conductance properties. Channels with three distinct unitary conductances (141, 108, and 70 pS) are observed in the A7r5 cells (15): two were observed in the human coronary cells (51 and 107 pS), and only one was observed in the pig coronary (59 pS) and rat mesentery (75 pS) cells. We showed previously that Cx43 comprises the 141- and 108-pS channels in the A7r5 cells, and it seems reasonable to speculate that Cx43 comprises the 59- and 75-pS channels observed, respectively, in the pig coronary and rat mesentery cells. We also demonstrated previously that Cx40 comprises the 70-pS channel observed in the A7r5 cells, and again it seems reasonable to speculate that Cx40 comprises the 51- and 107-pS channels observed in the human coronary cells. However, regardless of their connexin composition, the observed differences in unitary conductance indicate differences in permeability characteristics. Such differences are indicative of differences in permeability to anions vs. cations (41–43) and may have significant ramifications for tissue function (7, 22).

It was surprising, in view of the observed differences in connexin expression and unitary conductances, that each of the examined SMC types exhibited an increase in junctional conductance with 5-HT stimulation. As stated previously, an increase in junctional conductance can occur as a result of an increase in channel number, unitary conductance, open probability, or a combination of these possibilities. It is unlikely, in the 1- to 2-min response time, that channel number would increase or that a new connexin would be expressed, although this possibility cannot be unequivocably excluded. Our results indicate that the unitary conductances of the expressed channels did not change significantly, although their relative frequencies changed. Thus it is likely that the observed increases in G_j are explained by changes in open probability of one or more of the channel populations.

If our assumption regarding channel number is correct, then the increase in G_j observed in the mesentery and pig coronary cells must be explained by an increase in open probability of the 75- and 59-pS channels, respectively. This conclusion is supported by the observation that unitary conductance was essentially unaffected by 5-HT. In the human coronary and A7r5 SMCs multiple channel types are present, and their relative frequencies change between the control and 5-HT treatment conditions. By use of the human coronary cells for purposes of discussion, the relative frequency of the 107-pS channel population was reduced from 64% of the total events to nearly 0%. Such a change could reflect one of three mechanistic possibilities: *1*) decrease of P_o

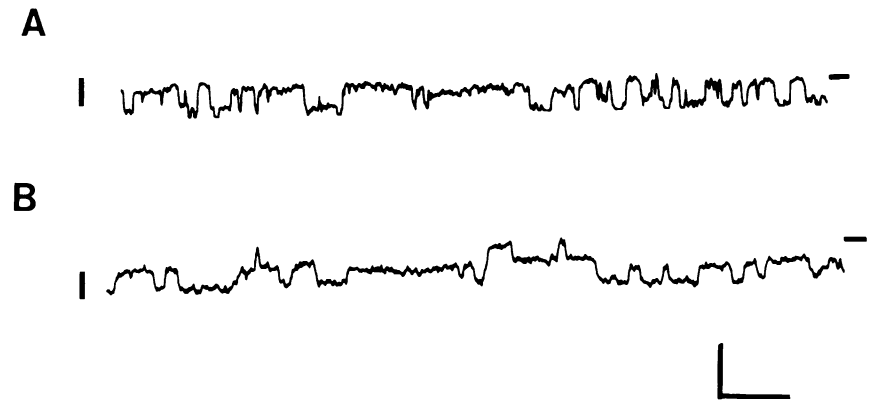

Fig. 6. Single-channel recordings from pig coronary (*A*) and rat mesentery (*B*) VSM cells in presence of 5-HT. Note in both cases that events of a single amplitude predominate. Channels open in downward direction. In both cases current trace from cell held at 0 mV (*dash*) is illustrated; the other cell was held at −50 mV. Vertical calibration = 10 pA; horizontal calibration = 2 s. Both records were filtered at 10 Hz.

to near 0%, *2*) increase of P_o to near 100%, or *3*) conversion of the 107-pS channel type into the 51-pS channel type (e.g., through phosphorylation). If P_o of the 107-pS channel decreased to near zero levels, then the open probability of the 51-pS channel must have simultaneously increased very dramatically to offset the loss of the 107-pS channel such that the net result would be the observed increase in G_j. Similarly, if the 107-pS channel were converted to the 51-pS channel type, then the loss of unitary conductance (107 to 51 pS) must have been offset by an increase in P_o of the 51-pS channel such that the net result would be the observed increase in G_j. Finally, if P_o of the 107-pS channel increased to nearly 100%, this change by itself could explain the 26% increase in G_j observed in this cell type, and no further change in P_o of the 51-pS channel would be required.

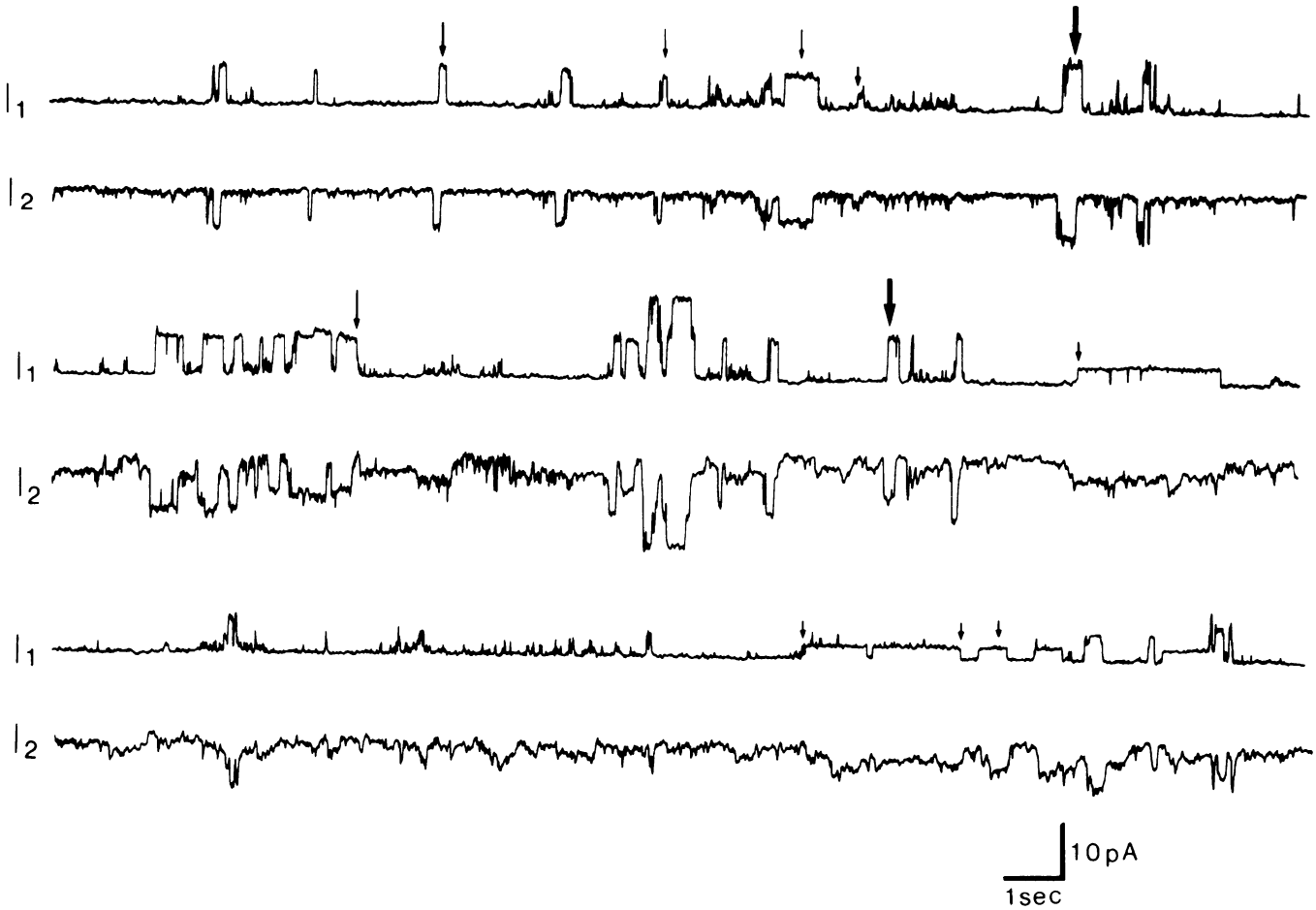

Fig. 7. Single-channel recording from human coronary smooth muscle cell pair after short-term exposure to 1 μM 5-HT. Gap junctional events can be recognized as transitions in 2 current traces (I_1 and I_2 for *cells 1* and *2*, respectively) that are equal in amplitude but opposite in polarity. Multiple channel conductances were evident (arrows of increasing length indicate events of increasing amplitude, which correspond to increasing conductances 70, 130, 160, and 180 pS). Some nonjunctional channel events are evident in this recording. Transjunctional potential difference was 40 mV with *cell 1* at 0 mV and *cell 2* at −40 mV. Channel opening occurs in upward direction in I_1 and downward direction in I_2. Record filtered at 30 Hz.

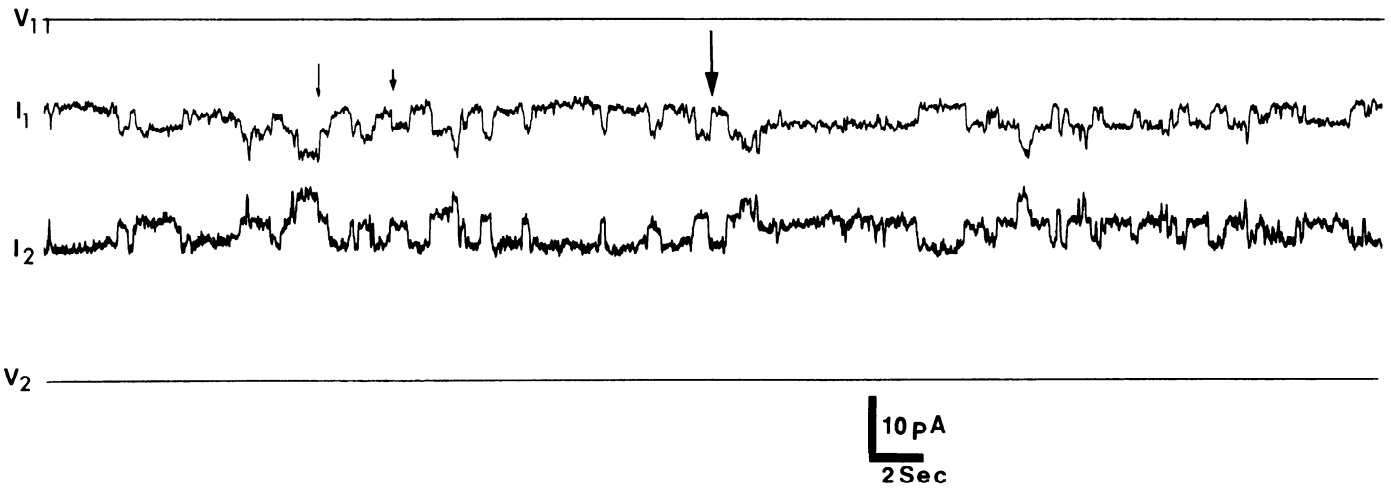

Fig. 8. Single-channel events observed in A7r5 cells during 5-HT treatment. Note presence of 70-, 108-, and 141-pS channel events (short, intermediate, and long arrows, respectively). Channel opening occurs in downward direction in I_1 and upward direction in I_2. *Cell 1* was held at 0 mV and *cell 2* at −40 mV. Record was filtered at 30 Hz.

Further experiments are required to resolve these possibilities, but the extraordinary changes in the relative frequency histograms indicate that it is highly unlikely that open probabilities for the two channel types were affected to exactly the same extent during the 5-HT treatment. Thus our data indicate that the observed increases in G_j in these four SMC types most likely reflect changes in open probabilities of the channels contributing to G_j. Furthermore, our data suggest that open probabilities of the various channel types were not uniformly affected during 5-HT treatment.

Direct measurement of P_o requires poorly coupled cell pairs, preferably with only one active channel but certainly with $G_j < \sim 1$ nS. Virtually all types of cells express sufficient gap junction protein that they are coupled by numerous functional channels and consequently G_j greatly exceeds 1 nS. As a consequence of these high G_j levels, there have been no direct measurements of P_o before and during activation of second messenger cascades. Our data strongly suggest that changes in P_o must occur during stimulation with 5-HT. Whether such changes reflect phosphorylation of the channel proteins remains to be proved; suffice it to say that both Cx43 and Cx40 are phosphoproteins (9, 20, 26, 28, 29, 37, 39, 40). For Cx43 there are several reports indicating that changes in phosphorylation state result in changes in the unitary conductance of the channel (23, 29, 39). Such changes cannot underlie the increase in G_j observed in the mesentery and pig coronary cells (where γ_j was stable) and are unlikely to be the sole explanation for the results obtained in the two cell types expressing multiple channel types as discussed above.

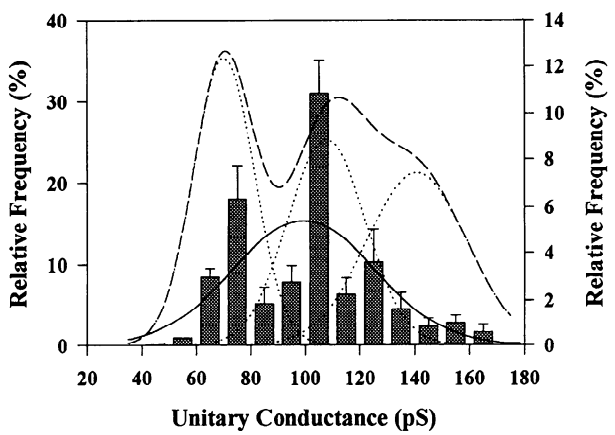

Fig. 9. Relative frequency of events-amplitude histograms of single-channel events observed in A7r5 cells during 5-HT treatment. A7r5 cells typically exhibit three channel populations with unitary conductances of 70, 108, and 141 pS. [To facilitate comparison, the composite fit (dashed line) and individual peaks (dotted line) for the control data are provided (25). These are referenced against the right axis.] During short-term exposure to 1 μM 5-HT there was a reduction in frequency of both 70- and 141-pS channel populations so that neither could be fitted as distinct populations. Mean of remaining peak, 99 ± 26 pS, is shifted relative to nontreated control (108 ± 14 pS), and SD for this peak is larger than for control situation. These differences suggest that 70- and 141-pS events are unresolved within 99-pS peak. Data were obtained from 10 cell pairs, 1,231 events, and were smoothed by 12% before fitting ($F = 167$, $r^2 = 0.965$). Error bars, SE.

The physiological significance of our findings must be viewed in the context of the diverse strategies for regulation of vascular smooth muscle function. Smooth muscle contractile function can be modulated through membrane potential-dependent mechanisms (e.g., stretch), membrane potential-independent mechanisms (e.g., receptor-mediated activation of intracellular second messenger systems), or a combination of these two. All gap junction channels mediate exchange of electrical signals. Although action potentials are infrequently noted in VSM, changes in VSM membrane potential (induced neurally by vasoactive agents or by stretch) spread electrotonically via gap junctions through and along the vessel wall (16) and result in coordinated changes of vessel diameter (34). Thus an increase of 30–70% in junctional conductance would facilitate rapid coordination of electrically mediated phenomena through the vessel wall and would enhance the coordinated response of vessels and consequently blood flow within the vessel bed by the resulting increase in space con-

stants. Gap junctions also mediate exchange of nonelectrical signals; of particular interest here are elements of second messenger cascades (e.g., inositol trisphosphate or adenosine 3′,5′-cyclic monophosphate). In light of the unique permeability profiles of gap junction channels composed of different connexins (41–43), the observed changes in open probabilities would be expected to have significant impact on the exchange of these signals and consequently on tissue function. For example, if the activity of channels that are permeant to anions were lost (despite an overall increase in junctional conductance), then the exchange of many second messenger molecules would be lost and the coordinated functions supported by that exchange would be lost. Evidence for polarity of dye tracer movement across myoendothelial junctions and for differences in the permeability of endothelial cell vs. SMC junctions has recently been presented (22). Our data indicate that these permeability differences may be dynamically regulated rather than fixed properties of the vessel wall cells.

In summary, our data suggest that VSM cells isolated from different vessels and species expressed different gap junction proteins and channels of different unitary conductances. During 5-HT stimulation each of the four cell types exhibited an increase in junctional conductance that was best explained by changes in channel open probability. However, our data suggest that open probabilities of the various channels found in these cells were not affected uniformly by 5-HT treatment. Thus variation in gap junction protein expression, channel function, and mechanism of response to common stimuli could contribute to observed differences in responsiveness of blood vessels from different organs and species to 5-HT.

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