Spontaneous Contractions in Elasmobranch Vessels In Vitro

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ABSTRACT Isolated vessels from four elasmobranchs, yellow stingray (Urolophus jamaicensis),
clearnose skate (Raja eglanteria), ghost shark (Hydrolagus novaezelandiae), and spiny dogfish
(Squalus acanthias), were examined for the presence of spontaneous contractions (SC). SC were
observed in otherwise unstimulated dorsal aortas (DA) from stingray and ghost shark, but not in
skate DA. Unstimulated ventral aortas (VA) did not exhibit SC. After treatment of VA with a
contractile agonist, SC appeared in stingray and skate but not ghost shark or dogfish. SC in
stingray VA were subsequently inhibited by either epinephrine (10–5 M) or indomethacin (10–4 M).
Agonist contraction also elicited strong SC in ductus Cuvier from stingray, but not from ghost
shark or dogfish. SC in dogfish hepatic portal veins (HPV) produced a rhythmic oscillation in
tension. The frequency of HPV SC was highest (~1 min–1) in intact veins and lower (~3 min –1) in
vein segments, indicative of a dominant pacemaker in the intact vessel. SC in HPV were de-
pressed during the first 30 min of hypoxia, but there was substantial recovery over an additional
30 min of hypoxia and complete recovery upon return to normoxia. Addition of 80 mM KCl com-
pletely inhibited HPV SC and lowered resting tone. These results show that SC are a common
feature of elasmobranch vessels and there appears to be a correlation between swimming behavior
and the propensity for SC. KCl inhibition of SC and tonus in HPV is highly unusual for vascular

Non-vascular smooth muscle is renowned for its
ability to contract spontaneously, whereas instances
of rhythmic activity in isolated blood vessels are
less well-characterized. Spontaneous rhythmic ac-
tivity, cyclic oscillations in smooth muscle contrac-
tion, has been reported in a few mammalian
arteries such as human coronary, pial, and umbilical
arteries (Ross et al., ’80; Elkhawad et al., ’83;
Stork and Cocks, ’94; Gokina et al., ’96), monkey
coronary artery (Ishii et al., ’85; Chemtob et al., ’92), rat and rabbit
aorta (Chemtob et al., ’92), rabbit basilar artery
(Young et al., ’87) and rat carotid (Hayashida et
al., ’87). Spontaneous activity has also been
-described, and perhaps best characterized in the
mammalian hepatic portal vein (Axelsson et al.,
’67; Sigurdsson et al., ’81; Schwietert et al., ’93).

There are a number of differences between spon-
taneous contractions of mammalian arteries and
the mammalian portal vein. First, spontaneous
contractions in arteries may only be evident in a
fraction of the arteries examined, i.e., from as few
as 3% of monkey coronary arteries (Ishii et al.,
’85) to 35% of rat thoracic aortas (Ishii et al., ’85;
Chemtob et al., ’92), or 50% of human pial arter-
ies (Gokina et al., ’96). Second, some arteries need
prior stimulation with a contractile agonist or a
potassium channel inhibitor before spontaneous
contractions appear (Young et al., ’87; Chemtob
et al., ’92; Trongvanichnam et al., ’96). Third, arterial contractions may be ini-
tiated and/or regulated by paracrine signals, ei-
ther from the endothelium or vessel wall (Chemtob
et al., ’92). Conversely, spontaneous contractions
are found in virtually all portal veins, and they

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are an indigenous attribute of the smooth muscle (Chemtob et al., '92).

The physiological significance of spontaneous contractions is unclear. Arterial spontaneity has been proposed to affect both blood flow and turbulence (Chemtob et al., '92) and in coronary arteries it may contribute to vasospastic angina pectoris (Ishii et al., '85). Femoral arteries from hypertensive rats exhibit spontaneous activity, whereas those from normotensive rats do not (Holloway and Bohr, '73). Portal vein contractions could enhance flow into the liver or reduce the tendency for blood stasis in this large vessel. It is not known whether differences in the spontaneous contractions between arteries and veins, are related to their respective physiological functions.

To our knowledge, spontaneous contractions have not been reported in isolated vessels from bony fish. We have not observed them in isolated rings from trout ventral aortas, afferent and efferent branchial arteries, coronary arteries, celiacomesenteric arteries, anterior cardinal veins, intestinal veins, or strips from ductus Cuvier (Olson and Meisheri, '89; Olson and Villa, '91; Conklin and Olson, '94). However, modest spontaneous contractions have been observed in coronary arteries from the mako shark, Isurus oxyrinchus, (Farrell and Davie, '91b) and we observed them during a survey of contractile properties of vessels from the stingray, Urolophus jamaicensis (Olson et al., unpublished observation). In the present study a variety of vessels from four species of elasmobranchs were examined to determine if spontaneous contractile activity is a general feature of elasmobranch vessels and to partially characterize these contractions.

**MATERIALS AND METHODS**

Yellow stingrays (Urolophus jamaicensis; n = 3, 300–450 g) were netted offshore near Christchurch, New Zealand by commercial fishermen. The animals were killed by an overdose of anesthetic (MS-222) in seawater and VA, DA, subclavian arteries (SA), IV, and strips of ductus Cuvier (DC) were removed and treated as noted previously.

Spiny dogfish (Squalus acanthias; n = 6, 60–75 g) were netted offshore near Christchurch, New Zealand by commercial fishermen and maintained in the Department of Zoology saltwater facility at the University of Canterbury. They were killed with a MS-222 overdose and VA, mesenteric arteries (MA), hepatic portal veins (HPV), and DC were removed and treated as noted previously.

The vessels were cleared of blood and adherent tissue and were cut transaxially into 2–3-mm-long rings. The rings were mounted on 280-μm diameter stainless-steel hooks and suspended in 20-ml smooth muscle chambers (Olson and Meisheri, '89) containing elasmobranch saline at 22 ± 2°C and aerated with an aquarium air pump. In Florida, tension was measured with Grass FT03C force-displacement transducers and recorded on a Grass polygraph. Sensitivity of the polygraph was set to detect changes as small as 5 mg. The analog output from the Grass was simultaneously digitized and archived on a personal computer. In Christchurch, temperature of the muscle baths was 14 ± 1°C. Vessel tension was measured with Ugo Basile 7004 isometric force transducers using Gould transducer amplifiers (model 13-4615-50) and Yokogawa series 4110 recorders.

The vessels were equilibrated to a resting tension of 150–250 mg (arteries) or 50–150 mg (veins) for at least 1 hr prior to experimentation. These tensions are within the optimal length-tension characteristics for the respective vessels (Olson, unpublished observation). A number of vessels exhibited spontaneous activity during the first 1–4 hr after placement in the muscle baths and they were examined directly. Other vessels were found to convert into spontaneous contractions only during treatment with a contractile agonist. Unstimulated vessels that did not exhibit spontaneity after 3 hr were randomly exposed to several contractile agonists including acetylcholine (Ach; 10^{-6} M), Carbachol (CBC; 10^{-4} M), arginine vasotocin (AVT; 10^{-6} M), endothelin-1 (ET-1, human form; 10^{-7} M) or the thromboxane A2 mimic, U-44069 (10^{-7} M) in an attempt to provoke spontaneous...
activity. In addition, the effects of hypoxia (aeration with nitrogen gas for ~60 min) and of elevated extracellular potassium chloride (80 mM KCl) plus hypoxia were examined in *Squalus* hepatic portal veins.

The intent of the present study was to survey a number of vessels in phylogenetically distinct elasmobranchs. *H. novazelandiae* is a holocephalian, the other three species are euselachians. *S. acanthias* is classified in the Squalioidea (de Carvalho, ’96) and the remaining two species are hypnosqualeans (*R. eglanteria*, Order Rajiformes and *U. jamaiicensis*, Order Myliobatiformes; McEachran et al., ’96). It was not possible to examine all vessels in all species due to anatomical differences and time limitations.

### Chemicals

Composition of elasmobranch saline used in Florida (Forster et al., ’72), in g liter⁻¹, was: NaCl, 16.33; KCl, 0.45; CaCl₂, 0.56; MgCl₂, 0.29; Na₂SO₄, 0.07; NaH₂PO₄, 0.12; NaHCO₃, 0.67; Urea, 21.0; TMAO, 7.99; glucose, 0.9 (colloid is not required in these experiments). In Christchurch, the phosphate and carbonate buffers were replaced with 0.72 g liter⁻¹ HEPES (acid form) and 1.82 g liter⁻¹ HEPES (sodium salt) to prevent precipitation of calcium salts (Olson, unpublished observation). U-44069 was a gift from Upjohn Pharmaceutical Company (Kalamazoo, MI), Endothelin-1 was purchased from Bachem Co. (Torrence, CA) All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

### Results

#### Stingray

All stingray vessels examined, VA, DA, IV, and DC, exhibited spontaneous activity sometime during an experiment (Fig. 1). These contractions were one of two types; a low-frequency, high-amplitude contraction in which force developed within 30 sec followed by an exponential decay in tension, or a rhythmic high-frequency, low-amplitude, and often oscillating tension.

Unstimulated VA did not exhibit spontaneous contractions. However, all VA examined (nine rings from three fish) exhibited low-frequency, and somewhat rhythmic (one every 6–12 min) contractions during constant exposure to either U-44069 (Fig. 1A) or endothelin-1 (not shown). Both ago-

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**Fig. 1.** Spontaneous contractions in stingray, *U. jamaiicensis*, ventral aorta (A), dorsal aorta (B), and ductus Cuvier (C, D). Arrow in A indicates application of 10⁻⁶ M U-44069 and initiation of spontaneous contractions in ventral aorta. Dorsal aortic rhythmicity (B) was independent of pre-existing stimuli but of low-amplitude (note scale in B). Arrow in C indicates removal of U-44069 (10⁻⁶ M) and decline in rhythmicity. High-frequency, low-amplitude contractions in ductus Cuvier (inset in D) were independent of pre-existing stimuli but disappeared during high-amplitude contractions. Multiple contractions forming a single high-amplitude contraction are shown in D.
nists initially produced a strong transient contraction that returned to baseline (Fig. 1A). The ensuing spontaneous contractions were extremely forceful (1–3 g in a 100–200-mg wet weight vessel), rapid in onset (< 20 sec to peak tension), and decayed exponentially over 3–6 min. Peak tension appeared to oscillate during these contractions, suggesting that each contraction was the sum of several (2–5) higher frequency “twitches.” Spontaneous contractions faded away after the agonist was washed off. Neither frequency nor tension was affected by storing the vessel for 4 days at 4°C. Epinephrine (10⁻⁵ M) applied 150 min after U-44069 inhibited spontaneous contractions. Indomethacin (10⁻⁴ M) inhibited spontaneous contractions and lowered baseline tension.

Spontaneous oscillating contractions began in unstimulated DA 1–1.5 hr after the vessels were placed in the chambers (Fig. 1B). DA contractions were weaker (20–50 mg) and more rapid (11–13 min⁻¹) in fresh vessels (n = 4) and became stronger (50–200 mg) and slower (1–2 min⁻¹) in vessels stored for 4 days at 4°C (n = 4). Spontaneous contractions disappeared when the vessels were stimulated with >10⁻⁷ M Ach but returned after the vessels were rinsed with fresh saline.

Two types of spontaneous contractions were observed in all (n = 8) ductus Cuvier (Fig. 1C,D). Weak (20–100 mg) and rapid (20–55 min⁻¹; average = 42 ± 8 min⁻¹) contractions with regular rhythm were present in unstimulated DC. These were either twitch-like, or appeared to have a sustained contraction interrupted by transient relaxations (Fig. 1D, inset). U-44069 produced a strong, transient contraction, as it did in the VA, and during exposure to U-44069, low-frequency, somewhat irregular rhythm (one every 3–10 min), and high-amplitude (1–3 g) contractions appeared (Fig. 1C). These contractions were similar in shape to the VA contractions and appeared to be the sum of a number of individual contractions. Although the frequency of these contractions decreased somewhat with time, they persisted for the duration of the experiment (4 hr). In some cases a sustained contraction occurred (Fig. 1D). All high-frequency, low-amplitude contractions disappeared during the onset of the low-frequency high-amplitude contractions and the former gradually reappeared as the vessel recovered from the latter. Frequency of the low-amplitude contractions did not change as the vessel recovered from the high-amplitude contraction. Epinephrine (10⁻⁵ M) greatly augmented the amplitude (> 400%) of the fast rhythmic contractions in DC.

Spontaneous contractions were observed in three out of six intestinal veins. These contractions were characterized by a relatively rapid onset (15–20 sec) and a slower recovery (45–60 sec) and occurred every 3–5 min. The tension developed was usually less than 100 mg and often appeared to be the result of several contractions fused together.

Skate

Spontaneous activity was not observed in unstimulated VA (n = 4 fish) or DA (n = 4 fish). In all VA and three out of four DA precontracted with U-44069, only slight (10–20 mg every 3–5 min) oscillations in tension were observed. Strong oscillating contractions (200–400 mg every 4–6 min) were observed in the fourth DA after U-44069 treatment. In this DA, 10⁻⁵ M epinephrine reduced baseline tension and decreased the amplitude of contraction by ~30% (Fig. 2A).

Ghost shark

Spontaneous contractions were observed in all unstimulated DA (n = 4; Fig. 2B) and SA (n = 2) but were not present in unstimulated VA, IV or DC, nor were they induced by stimulating these vessels with U-44069 or ET-1 (N = 4, all groups). Contraction frequency in DA and SA was variable from vessel to vessel and generally increased from one contraction every 15–25 min shortly after the vessels were placed in the muscle baths to one every 2–10 min 2–3 hr later.

Spiny dogfish

Spontaneous contractions were observed in unstimulated MA and HPV from all dogfish, but were not evident in DC and were erratic to nonexistent in VA (n = 4). MA contractions were similar to those observed in the skate and occurred approximately once every 2–5 min. HPV contractions were striking in both intensity and frequency and they were examined in greater detail.

Spontaneous contractions recorded from a segment of the HPV (usually one-fourth to one-third of the vessel length; Fig. 3A) occurred 3–4 times every 10 min and lasted approximately 2–3 min. These contractions were spike-shaped and high-amplitude. Resting tension between the spikes was relatively stable. Spontaneous contractions recorded from an intact HPV (Fig. 3B) were more frequent (9 min⁻¹) and there was no quiescent period between contractions. All preparations (n = 4) exhibited steady, spontaneous activity for 36 hr or more.
Hypoxia, produced by aerating the vessel bath with nitrogen gas, initially depressed the magnitude of spontaneous contractions and reduced the resting tone between contractions (n = 4; Fig. 4A). Spontaneous contractions returned 30–35 min into continuous hypoxia, albeit at a lower frequency, and the amplitude of contraction was enhanced. Upon return to air, there was a rapid increase in resting tension to above pre-hypoxia level and the frequency of spontaneous contractions was restored. Resting tension gradually fell to pre-hypoxia levels over the ensuing hour. Addition of 80 mM KCl abolished all spontaneous contractions and produced a rapid fall in resting tone (Fig. 4B). Hypoxia did not have any additional effect, however, upon return to normoxia there was a single contraction and resting tone was increased but did not return to pre-KCl levels. Spontaneous activity was restored within an hour after the vessel was rinsed with low KCl buffer (not shown).

**DISCUSSION**

It is evident from the present study that spontaneous contractions occurred in both arteries and veins of a variety of elasmobranchs. Overall, these contractions were quite variable with regard to shape, frequency and dependency on pre-existing stimuli. However, within a given vessel the contractions were surprisingly uniform. Thus it seems likely that spontaneous contractions perform useful functions and that these functions may vary both with the location of the vessel and with other physical and physiological characteristics of the fish.

The high-frequency, low-amplitude and low-frequency high-amplitude contractions of the ductus Cuvier (Fig. 1C,D) appeared to result from two
distinct processes. The high-frequency contractions were probably due to small pieces of sinus venosus that remained attached to the ductus, as it was technically difficult to remove an intact segment of ductus that was free from all adjacent tissue. Epinephrine increased the force of the high-frequency contractions, consistent with its effect on cardiac muscle and opposite to the general inhibitory effect of this catecholamine on many stingray blood vessels (Olson et al., unpublished observation). Conversely, the high-amplitude contractions were most likely due to smooth muscle because it is doubtful that there was enough sinus tissue adhering to the ductus to produce these elevated tensions. High-amplitude contractions of the DC, like those of the VA, appeared to result from the summation of a number of individual contractions (Fig. 1D). This may be similar to spontaneous contractions of the rat hepatic portal vein that result from bursts of action potentials (Axelsson et al., '67). Ductal contractions may also be relevant to swimming behavior patterns as they were observed in the stingray but not in the more active ghost shark or dogfish (it was not possible to obtain tissue from skate).

The most striking spontaneous contractions were found in the dogfish hepatic portal vein (Fig. 3). The highest frequency contractions were observed in intact vein, whereas the frequency was considerably lower in shorter segments of the vessel. This suggests that, while much of the vessel is capable of spontaneously contracting, in vivo there is a single pacemaker that drives the entire vessel. Pacemaker frequency also appears to be an invariant feature of the specific segment of vessel. Both the intact vessel and isolated segments maintained their respective frequencies (and the smooth muscle developed the same tension) for over 36 hr in vitro.

Dogfish hepatic portal veins are similar to mammalian portal veins in the consistency in which spontaneous contractions are observed (~100%) and in their ability to contract independent of prior agonist stimulation. Dogfish portal veins (Fig. 4A) like mammalian portal veins (Ebeigbe et al., '80; Sigurdsson et al., '81) were also sensitive to hypoxia. However, in the mammalian hepatic portal vein the frequency of spontaneous contractions initially increased during hypoxia and shortly thereafter tension development was virtually abolished, and it remained so as long as the hypoxia was maintained (Sigurdsson et al., '81; Sigurdsson and Grampp, '81). This is unlike the dogfish veins in which both the resting tension and spontaneity were initially depressed and then began to recover within 20 min. Furthermore, dogfish veins continued to recover, even though the hypoxia was maintained for an additional 40 min (Fig. 4A). The recovery of spontaneous contractions during hypoxia did not appear to be due to a general rundown of the smooth muscle and the resultant inability to maintain membrane potential because upon restoration of normoxia there was an immediate overshoot of resting tension.

Perhaps the greatest disparity between dogfish and mammalian hepatic portal veins was in their response to elevated potassium chloride. In mammalian veins, 50–128 mM KCl depolarized muscle cells and produced a tonic, sustained contraction (Axelsson et al., '67; Ebeigbe et al., '80; Schwietert et al., '93), whereas in the dogfish it inhibited both resting tone and prevented spontaneous activity in the presence of normoxia or hypoxia (Fig. 4B).
As potassium-invoked contraction is the typical, and expected, response of vascular smooth muscle in both mammals and fish (Olson and Meisheri, '89), the relaxation of dogfish portal veins with 80 mM KCl is remarkable. In fact, potassium-inhibition of portal veins is more in line with the inhibitory effect of elevated potassium on cardiac muscle.

It is not too surprising that we observed spontaneous contractions in a number of vessels from all four species of elasmobranchs, even though there are very few reports of these contractions in the literature. Our study encompassed a variety of vessels not routinely investigated in elasmobranchs. Heretofore the majority of studies on isolated vessels of elasmobranchs have focused on a few specific vessels such as the ventral aorta and its branches (Bjenning et al., '92; Evans, '92; Evans et al., '96; Hamano et al., '98) or coronary arteries (Farrell and Davie, '91a,b). (However, spontaneous contractions were similarly absent from the coeliac artery of Triakis scyllia; Hamano et al., '98.) We found that spontaneous contractions in Squalus VA were erratic and weak and it is not surprising that they would be missed unless one was specifically looking for them. Similarly, Farrell and Davie ('91b) observed spontaneous contractions in only 2 of 20 coronary artery rings from mako sharks and in these the active tension was only around 12% of the baseline tension.

In our studies, we were also careful to allow sufficient time, often in excess of 2–3 hr, for spontaneity to develop, and we provided a backdrop of conditions, both unstimulated and agonist-induced tone, for the vessels. Because the focus of most other studies of elasmobranch vessels has been the concentration-dependent response to various agonists and antagonists, understandably there is usually not sufficient opportunity for spontaneous contractions to develop, either prior to treatment, or at any single treatment dose. Farrell and Davie ('91b) noticed this in the mako shark. They (Farrell and Davie, '91b) found that spontaneous contractions could be invoked in mako coronary arteries by low doses of prostaglandin F$_2$ and that at higher prostaglandin concentrations the spontaneous contractions were masked by a tonic elevation in tension. It is quite likely that there have been a number of concentration-response studies in which, due to the design of the experiment, there was insufficient time for sustained rhythmic activity to develop.

In spite of the prevalence of spontaneous contractions in elasmobranch vessels, this trait may not be common in non-elasmobranch fish. Spontaneous contractions have not been observed in a variety of arterial and venous vessels from trout, Oncorhynchus mykiss (Olson and Meisheri, '89; Olson and Villa, '90; Small et al., '90; Conklin and Olson, '94), gar (including hepatic vein), Lepisosteus spp. (Conklin et al., '96), or bowfin, Amia calva (Russell, Jones and Olson, unpublished observation). Spontaneous contractions were observed, however, in 2 of over 80 dorsal aorta rings from 20 hagfish, Eptatretus cirrhatus, but they were not present in ventral aorta, segmental arteries, posterior cardinal veins, or intestinal veins (Forster and Olson, unpublished observation). Similarly, they have not been observed in either ventral or dorsal aortic rings from the lamprey, Petromyzon marinus (Russell, Jones and Olson, unpublished observation). It is possible that conditions in the above studies were not optimal for spontaneous contractions to develop and that rhythmic contractions are common in all fish, although this seems unlikely. Rather, it appears that elasmobranch vessels are more prone to develop spontaneous contractions than either cyclostomes or bony fish. It is also difficult to associate spontaneous contractions with the low blood pressures in elasmobranch fish. Certainly VA and DA pressures in elasmobranchs fall into the low end of the range of values recorded in teleosts, but they are roughly double those found in cyclostomes (Bushnell et al., '92).

There are a number of similarities between spontaneous contractions in elasmobranch and mammalian arteries. First, spontaneous contractions are not present in all arteries, or if present, they are not necessarily found in every vessel of that type. Second, in a number of elasmobranch vessels spontaneous contractions are not evident until after the vessel is pretreated with a contractile agonist. Third, paracrine signaling may be involved in some instances, as indomethacin inhibits spontaneous contractions in both the rat thoracic aorta (Chentob et al., '92) and stingray VA. It is, therefore, quite likely that the variety of mechanisms involved in excitation contraction coupling in elasmobranch arteries are similar to those well characterized in mammals. The differences between elasmobranch and mammalian portal veins are worth further investigation.

The present study suggests that the widespread occurrence of spontaneous contractions in elasmobranch vessels is not anecdotal and that these contractions may perform one or a variety of physiological functions. Spontaneous contractions of arteries were, in general, more prevalent in the
stingray and skate (albeit of lower magnitude) than they were in either the ghost shark or spiny dogfish. This may be related to the more sedentary behavior patterns of the batooids, in which skeletal muscle pumps would be predicted to have diminished utility. VA contractions were generally spike-like and of considerable amplitude. This type of contraction may augment or assist the pumping ability of the heart. In contrast, DA contractions were oscillatory and low amplitude. These would not be expected to provide an appreciable boost to arterial pressure, but may prevent local stasis or decrease the tendency for red blood cells to settle out in this long, horizontal vessel. Realization of the benefits of DA rhythmic activity would also be emphasized in the less active species. Perhaps this point is also reflected in the fact that the frequency of rhythmic contractions was highest in the stingray, lowest in the ghost shark and contractions were non-existent in the dogfish dorsal aorta. Clearly additional studies on other vessels and different species are necessary to resolve these issues.

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LITERATURE CITED


