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CONTRACTILE PROPERTIES OF THE ELASMOBRANCH RECTAL GLAND

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Summary

The importance of the rectal gland in elasmobranch osmoregulation is well established. The rate of secretion by the gland is under the control of a variety of secretagogues and inhibitors. Early morphological work suggested that a band of smooth muscle cells surrounds the periphery of the shark rectal gland between the secretory tubules and the connective tissue capsule. To confirm the presence of the muscle ring, we examined histological sections from two species of shark, Squalus acanthias and Carcharodon carcharias, and from the stingray Dasyatis sabina and stained sections from S. acanthias with the actin-specific ligand phalloidin. In all three species, a distinct band of what appeared to be smooth muscle cells was evident, and the putative muscle ring in S. acanthias stained specifically with phalloidin. Moreover, isolated rings of rectal gland tissue from S. acanthias constricted when acetylcholine or endothelin was applied and responded to nitric oxide with an initial dilation, followed by a more substantial constriction. Subsequent addition of porcine C-type natriuretic peptide dilated the rings, but two prostanoids (carbaprostacyclin and prostaglandin E1) did not change ring tension significantly. The rings did not respond to the endothelin-B-specific agonist sarafotoxoin S6c, suggesting that the response to endothelin was mediated via endothelin-A-type receptors. Our data confirm the presence of a smooth muscle ring in the periphery of the elasmobranch rectal gland and demonstrate that the gland responds to a suite of smooth muscle agonists, suggesting that changes in the dimensions of the whole rectal gland may play a role in its secretory function.

Key words: elasmobranch, shark, Squalus acanthias, Carcharodon carcharias, stingray, Dasyatis sabina, rectal gland, osmoregulation, smooth muscle receptor.

Introduction

The elasmobranch rectal gland secretes a plasma-hypertonic solution that is generally considered to play a major role in osmoregulation in these marine fishes (e.g. Shuttleworth, 1988), although osmoregulation continues after extirpation of the gland (e.g. Burger, 1965; Evans et al., 1982). In the spiny dogfish Squalus acanthias, the rate of secretion is 47μl 100g−1 h−1 and the fluid Na+ plus Cl− concentration totals nearly 1000 mmol l−1, approximately double that in the plasma (Burger and Hess, 1960; Burger, 1962). The ionic transport mechanisms mediating the production of this secretion have been extensively studied (for reviews, see Riordan et al., 1994; Hazon et al., 1997; Silva et al., 1997; Karnaky, 1998), and it is clear that the net ionic secretion is via a basolateral Na+/K+2Cl− cotransporter (driven by Na+/K+-ATPase on the same membrane; Forrest, 1996) and an apical Cl− channel homologous to the mammalian cystic fibrosis transmembrane conductance regulator (CFTR; Marshall, 1991).

Secretion of fluid from the shark rectal gland is controlled by a variety of neural and endocrine factors. Early work demonstrated that vasoactive intestinal peptide (VIP) was a potent secretagogue for the isolated, perfused rectal gland of S. acanthias (Stoff et al., 1979), and VIPergic nerves were localized in the gland ‘extending from the outer fibromuscular (underline ours) capsule towards the excretory duct, in close proximity to secretory tubules’ (Stoff et al., 1988). The generality of the role of VIP in controlling rectal gland secretion has been challenged (Shuttleworth, 1988), because the glands of Scyliorhinus canicula did not respond to VIP (Thorndyke and Shuttleworth, 1986; Anderson et al., 1995). An endogenous, stimulatory peptide was isolated from the intestine of this species (Shuttleworth and Thorndyke, 1984) and has been found to be identical to the tachykinin scyliorhinin II (Anderson et al., 1995). At least in S. acanthias, natriuretic peptides (NPs) enhance gland secretion both by stimulation of the release of VIP (Silva et al., 1987) and by direct activation of NP receptors (NPRs) on the epithelial cells (Karnaky et al., 1991). These receptors are now known to be the NPR-B type (Gunning et al., 1993), and the shark homologue has been cloned from the rectal gland (Aller et al., 1999). Neuropeptide Y and somatostatin both inhibit rectal gland secretion in S. acanthias (Silva et al., 1990; Silva et al., 1993), as does bombesin but via release of somatostatin (Silva et al., 1990). It appears that metabolic activity in the gland cells themselves may play a regulatory role, since adenosine has
been shown to inhibit secretion at low concentrations (10 nmol l\(^{-1}\) to 1 \(\mu\)mol l\(^{-1}\)) and to activate it at higher concentrations (10–100 \(\mu\)mol l\(^{-1}\); Forrest, 1996).

Salt secretion by the rectal gland in vivo may also be controlled by perfusion limitations in the gland itself or in the posterior mesenteric artery, which supplies the gland. Perfusion of isolated glands from S. acanthias and S. canicula (at in vivo flow rates) with norepinephrine reduced efferent perfusate flow and salt secretion by the gland (via \(\alpha\)-receptors) in a concentration-dependent manner, suggesting that the vasculature within the gland was constricted (Shuttleworth, 1983; Shuttleworth and Thompson, 1986). Since the levels of norepinephrine used approached in vivo concentrations, Shuttleworth (Shuttleworth, 1988) suggested that the rectal gland may be tonically constricted when unstimulated. Importantly, two known secretagogues of S. acanthias rectal gland salt secretion, adenosine and VIP, reversed the norepinephrine-mediated vasoconstriction (at least in S. acanthias glands), suggesting that some of the effects of these secretagogues may be mediated by intragland perfusion changes, not solely by stimulation of ionic transport steps (Shuttleworth, 1983). In addition, we have recently found that the anterior mesenteric artery in S. acanthias responds to nitric oxide (NO) and expresses receptors for acetylcholine (ACh), endothelin (ET), NPs and prostaglandins (PGs; D. H. Evans, in preparation), so one might hypothesize that the posterior mesenteric artery may also be controlled by a suite of vasoactive signalling agents, which could modify rectal gland function by changes in perfusion of the gland.

Reappraisal of the morphology of the rectal gland suggests that volume changes in the gland itself may play some role in secretion. In an early light- and electron-microscopic study of the rectal gland of S. acanthias, Bulger (Bulger, 1963) described a circumferential ‘inner muscle layer’ between the secretory tubules and the connective tissue layer of the outer capsule, to which Stoff et al. (Stoff et al., 1979) referred. Our recent studies have delineated receptors in S. acanthias aortic vascular smooth muscle for NPs (Evans et al., 1993), ACh (Evans and Gunderson, 1998a), ET (Evans et al., 1996) and PGs (Evans and Gunderson, 1998b) and have shown that the vascular smooth muscle is also sensitive to NO (Evans and Gunderson, 1998b). It therefore seemed appropriate to re-examine the structure of the elasmobranch rectal gland and measure the sensitivity of isolated rings of rectal gland tissue to these substances to test the hypothesis that the gland itself is contractile and responds to signalling agents that control smooth muscle tension in other tissues.

Materials and methods

Spiny dogfish (Squalus acanthias L., approximately 2–5 kg) were captured in Frenchman Bay, Maine, USA, and maintained in running sea water until killed by pithing through the snout. The rectal gland was removed by blunt dissection and either fixed immediately for histology (Bouin’s for 24 h) or cut into 2–3 mm thick cross-sectional rings approximately

Fig. 1. Sections of rectal gland tissue from Squalus acanthias (A), Carcharodon carcharius (B) and Dasyatis sabina (C). The putative muscle layer (marked with an asterisk) stains green and contains elongate nuclei. Scale bars, 100 \(\mu\)m. See text for details.
6 mm diameter for tension measurements. A rectal gland from a single Atlantic stingray (*Dasyatis sabina*, approximately 1 kg), caught by hook and line near Cedar Key, Florida, USA, was prepared and fixed in the same way. In addition, a rectal gland was removed from a great white shark (*Carcharodon carcharias*, 2.73 m) that had been caught off the east coast of Florida by a commercial fisherman and transferred to the University of Florida in ice. This rectal gland was fixed in 10% neutral buffered formalin (NBF) for 24 h.

For histological examination, rectal gland tissue that had been in Bouin’s or NBF fixative for 24 h was dehydrated in a graded ethanol series and embedded in paraffin. Sections 5 μm thick were cleared, rehydrated and stained with a modified Trichrome of Harris (Humason, 1972). To characterize further the putative muscle layer, thick (500 μm) frozen sections of *S. acanthias* rectal gland tissue were fixed with 3.7% formaldehyde plus 0.5% Triton X-100 in elasmobranch Ringer and stained for F-actin with rhodamine-phalloidin (1 μg ml⁻¹). Control sections were treated in the same manner, but did not have rhodamine-phalloidin applied. The fluorescent staining was imaged using an Olympus Fluorview point-scanning confocal microscope.

To test the effect of putative signalling agents, rings of rectal glands were mounted in elasmobranch Ringer’s solution in thermo-jacketed chambers (12 °C) and maintained at approximately 200 mg tension as described for aortic rings of the same species (Evans and Gunderson, 1998b). Our preliminary experiments determined that the rectal gland rings were most responsive at this tension. Tension was recorded by WPI strain transducers connected through a Biopac MP100WS data-acquisition system (using AcqKnowledge III software) to a Macintosh Powerbook 140 computer. After the rings had reached a stable tension, putative agonists were added cumulatively to the experimental bath in increments totalling less than 4% of the initial volume.

Solutions of acetylcholine (ACh, Sigma), human endothelin-1 (ET-1; American Peptide), sarafotoxin S6c (SRX S6c; American Peptide), porcine C-type natriuretic peptide (pCNP; Peninsula Labs), eel atrial natriuretic peptide (eANP; Peninsula Labs), carbaprostacyclin (CPR; Cayman Chemicals) and prostaglandin E₁ (PGE₁; Cayman Chemicals) were solubilized as described previously (Evans et al., 1996; Evans and Gunderson, 1998b) and stored at −70 °C until use. A saturated NO solution was prepared in distilled water as described previously (Evans et al., 1996; Evans and Gunderson, 1998b) and stored at 70 °C until use. A saturated NO solution was prepared in distilled water as described previously (Evans et al., 1996; Evans and Gunderson, 1998b). Initial experiments consisted of the sequential addition of the following: ACh (0.1 μmol l⁻¹), ET-1 (0.1 μmol l⁻¹), NO (8.4 μmol l⁻¹), pCNP (0.1 μmol l⁻¹), CPR (1.0 μmol l⁻¹) and PGE₁ (1 μmol l⁻¹) to a given ring. In subsequent experiments, to differentiate between ETₐ and ETₐ receptors, paired rings were exposed to 0.1 μmol l⁻¹ of either ET-1 or SRX S6c. This was followed by the addition of either 0.1 μmol l⁻¹ eANP or 0.1 μmol l⁻¹ pCNP to differentiate between NPRₐ and NPRₐ receptors. Specific concentrations of all agonists were chosen because our earlier studies had determined that they produced near-maximal responses in shark vascular smooth muscle (e.g. Evans et al., 1993; Evans et al., 1996; Evans and Gunderson, 1998b).

These protocols conformed to NIH Guidelines and were approved by the IACUC at Mount Desert Island Biological Laboratory.

All data are expressed as mean ± s.e.m. (N). Tension changes were compared with zero change using Prism (GraphPad Software) and accepted as significant at P < 0.05 (Student’s t-test, two-tailed).

**Results**

Cross sections of rectal glands from *S. acanthias*, *C. carcharias* and *D. sabina* (Fig. 1) all show a distinct band of elongated cells situated between the glandular tissue and the outer connective tissue of the capsule, in the same position as the band of ‘inner muscle’ described by Bulger (Bulger, 1963). Cells making up this band are characterized by elongate nuclei, suggesting muscle tissue (e.g. Telford and Bridgman, 1995). Rhodamine-phalloidin produced a fluorescent band in the same circumferential region as the putative muscle band in *S. acanthias* (Fig. 2). The control rings showed no autofluorescence (data not shown).

The rings of rectal gland tissue from *S. acanthias* responded to a suite of vasoactive substances. ACh and ET-1 both produced significant constriction (Figs 3, 5), but ET-1 was much more effective. The ETₐ-specific agonist SRX S6c did not constrict the rings, in contrast to ET-1, which stimulated substantial constriction in paired experiments (Fig. 6). Nitric oxide produced a biphasic response with an initial relaxation followed by a more significant constriction (Figs 4, 5). Porcine CNP produced significant relaxation of the rings in our initial experiments (Figs 3, 5); however, we found that eel ANP was a more potent relaxant than pCNP in paired experiments (Fig. 6). Neither the stable prostacyclin analogue CPR nor prostaglandin PGE₁ produced significant changes in tension of the rectal gland rings (Figs 3, 5).

**Discussion**

Our data corroborate earlier work (e.g. Bulger, 1963; Stoff et al., 1979) that described a band of smooth muscle fibres surrounding the the rectal gland of *S. acanthias*, just below the capsule (Figs 1, 2). In fact, the band is also present in the rectal gland of both the great white shark and the Atlantic stingray, suggesting that the muscle may be a common feature of most elasmobranch rectal glands. The fact that the band could be localized with phalloidin (Fig. 2) strongly suggests that the tissue contains muscle cells since phalloidin binds to actin microfilaments (e.g. Khayat et al., 2000).

Both the neurotransmitter acetylcholine and the peptide endothelin significantly constricted rings of rectal gland tissue (Figs 3–5), corroborating our earlier work on vascular smooth muscle from this species (Evans et al., 1996; Evans et al., 1998a; Evans, 2001). As for vascular smooth muscle, ET-1 was a much more effective constrictor than ACh. In fact, ET
is now considered to be the most potent smooth muscle constrictor in mammals (e.g. Miyauchi and Masaki, 1999), and this seems also to be the case in the lower vertebrates. Since the elasmobranch rectal gland is derived from intestinal tissue (Hoskins, 1917), it is important to note that ET has also been shown to constrict smooth muscle from the mammalian

![Image](image_url)

Fig. 2. Typical localization pattern of rhodamine-phalloidin in a rectal gland section from *Squalus acanthias*. A has been overexposed and reveals the intense staining of the outer layer of the gland and the weaker labelling of the internal tubules. B shows the same field underexposed and demonstrates the presence of spindle-shaped stained elements in the outer layer. At higher magnification (C), these elements appear similar to smooth muscle cells. Scale bars: A, 10µm; C, 20µm.

![Graph](graph_url)

Fig. 3. Copy of the computer tracing from a single experiment showing the ‘typical’ response to cumulative addition of 0.1 mmol l\(^{-1}\) acetycholine (ACh), 0.1 µmol l\(^{-1}\) human endothelin (ET-1), 8.4 µmol l\(^{-1}\) nitric oxide (NO), 0.1 µmol l\(^{-1}\) porcine C-type natriuretic peptide (pCNP), 1 µmol l\(^{-1}\) carbaprostacyclin (CPR), a stable agonist of prostacyclin (see Results), and 0.1 µmol l\(^{-1}\) prostaglandin E (PGE\(_1\)) to an isolated ring of rectal gland tissue from *Squalus acanthias*. 
Elasmobranch rectal gland smooth muscle receptors

The specific cholinergic receptor involved in the response to ACh is undetermined, but our earlier work on the ventral aorta of *S. acanthias* suggested that an M₃-type muscarinic receptor was expressed in that tissue (Evans and Gunderson, 1998a).

Endothelin receptors are generally characterized (Huggins et al., 1993) by their sensitivity to SRX S6c (specific for ET₄B) versus ET-1 (non-specific; both ET₄A and ET₄B). In mammals, the dominant receptor appears to be of the ET₄A type in arterial vessels (e.g. Levin, 1996), but the ET₄B receptor mediates constriction in veins and also in some arterial preparations (e.g. Moreland et al., 1992; Seo et al., 1994; Teerlink et al., 1994; White et al., 1994). The ventral aorta (and gill) of *S. acanthias* expresses an ET₄B-type receptor (Evans et al., 1996; Evans and Gunderson, 1999), but the absence of a response to SRX S6c in the rectal gland (Fig. 6) suggests that only ET₄A-type

Fig. 4. Tracings from four experiments showing the biphasic response to 8.4 μmol l⁻¹ nitric oxide (NO) addition to the rectal gland rings from *Squalus acanthias*. 
receptors are expressed in this tissue. Both ET\textsubscript{A} and ET\textsubscript{B} receptors are expressed in mammalian intestinal tissue, although ET\textsubscript{B} receptors appear to predominate (Masaki, 1993).

The source of ACh and ET that may stimulate constriction of the rectal gland is unknown at present. The elasmobranch rectal gland is innervated (Holmgren and Nilsson, 1983; Chipkin et al., 1988; Stoff et al., 1988), but these neurons have been described as VIP- or bombesin-containing (Donald, 1998), rather than cholinergic. However, we have described cholinergic receptors in three vessels from \textit{S. acanthias} (Evans and Gunderson, 1998a; D. H. Evans, in preparation), and there is no indication that any shark systemic vessels are innervated (Donald, 1998), so the role of cholinergic receptors in elasmobranch vasculature and rectal gland remains unclear.

The source of the ET that may constrict the rectal gland is also unknown, but may not be merely the vascular endothelial cells. Endothelin has been shown to be synthesized by a variety of tissues including the mammalian central nervous system and intestine (e.g. Masaki, 1993; Stjernquist, 1998). Immunoreactive ET (antibodies raised against the mammalian peptide) has been localized in both teleost and elasmobranch gills (Zaccone et al., 1996) and in the central nervous system of an agnathan \textit{Lampetra japonica} and a teleost \textit{Oryzias latipes} (Kasuya et al., 1991). Immunoreactive ET also has been described in molluscs, insects and the protochordate \textit{Ciona intestinalis}, suggesting a very ancient origin of this polypeptide family (Kasuya et al., 1991). It would be of interest to determine whether rectal gland tissue synthesizes ET.

The biphasic response to NO application is unusual. NO is the well-defined endothelium-derived relaxing factor, but it is now known to be produced by a variety of cells, most notably for this study, neurons and vascular smooth muscle (e.g. Sase and Michel, 1997; Shimpo et al., 2000; Wakabayashi et al., 2000). NO has not been shown to be constrictory in mammalian smooth muscle, but we have found significant constriction in \textit{S. acanthias} vascular smooth muscle (Evans and Gunderson, 1998b; D. H. Evans, in preparation) and in the ventral aorta of the hagfish \textit{Myxine glutinosa} (Evans and Harrie, 2001). The aorta of the lamprey \textit{Petromyzon marinus} responded to NO with an initial constriction followed by a more significant relaxation, but the aorta of the eel \textit{A. rostrata} dilated when NO was applied (Evans and Harrie, 2001).

The mechanisms underlying these NO-induced constrictions are unknown, but it is possible that they are mediated by an interaction between NO and superoxide to produce the reactive peroxynitrite ion (e.g. Beckman and Koppenol, 1996). We have found (Evans and Hagen, 2000) that 50\% of the NO-induced constriction of the \textit{S. acanthias} aortic ring could be inhibited by pre-treatment with the superoxide dismutase mimetic Tempol, which would reduce the intracellular concentrations of superoxide (and thereby peroxynitrite; Nilsson et al., 1989). Nitric oxide synthase (which mediates the production of NO from L-arginine) has been localized in the central and peripheral nervous system of a variety of teleost, elasmobranch and even agnathan fishes (e.g. Schober et al.,
1994; Bruning et al., 1996; Funakoshi et al., 1997; Holmqvist and Ekstrom, 1997; Karila et al., 1997; Cioni et al., 1998), but it has not been localized in the elasmobranch rectal gland to date.

Not surprisingly, pCNP dilated the rectal gland rings (Fig. 3). Shark CNP has been cloned from S. acanthias heart (Schofield et al., 1991), but it is not available, and our earlier studies demonstrated that pCNP is just as dilatory when applied to S. acanthias aortic rings as the homologous peptide (Evans et al., 1993). Interestingly, when the action of eANP was compared with that of pCNP in paired experiments, eANP was much more potent; indeed, the CNP-mediated dilation was not significant in these experiments (Fig. 6), which were performed a year later. We assume that population differences account for this discrepancy.

This apparent sensitivity to an ANP might suggest that an NPR-A receptor is expressed in the contractile tissue of the rectal gland, because ANP is relatively specific for NPR-A receptors, while CNP is very specific for NPR-B receptors (e.g. Takei, 2000). However, attempts to isolate an ANP or its mRNA in elasmobranch heart and brain have failed (Takei, 2000), and only NPR-B has been cloned from S. acanthias rectal gland (Aller et al., 1999), so it seems more likely that, in our experiments, ANP is stimulating an NPR-B receptor because of relatively high concentrations of the A-type natriuretic peptide. Pharmacological or molecular techniques will have to be employed to determine the actual NP receptor subtype, but it is clear that NPs can produce dilation of the elasmobranch rectal gland. The source of the peptide is unknown, but CNPs are produced by the elasmobranch heart and circulate in the blood (Takei, 2000).

The absence of a significant response to either prostanoid (Figs 3, 5) is surprising, since both CPR and PGE1 dilated the ventral aorta of S. acanthias and are produced by that tissue (Evans and Gunderson, 1998b). In addition, our more recent studies have demonstrated that both the anterior mesenteric artery and posterior mesenteric vein are also dilated by one or both of these prostanoids (D. H. Evans, in preparation). In any event, our data suggest that receptors for prostanoids may be absent from the contractile tissue of the rectal gland, in contrast to vascular smooth muscle in this species. Alternatively, the responses may have been below the detection limit of this protocol. In fact, the responses to any of the agonists should be considered to be minimal, since both the retaining wire and the wire connecting the tissue to the strain transducer were threaded through the gland lumen rather than just below the muscle layer. Thus, the magnitude of any contractions was reduced by spongy, glandular tissue. Nevertheless, the responses to putative signalling agents were significant in all cases, except for the prostanoids.

Although we have not localized specific receptors to the muscle band that is visualized in Figs 1 and 2 by immunohistochemistry or in situ hybridization, our data have demonstrated that the rectal gland of S. acanthias responds to smooth muscle signalling agents. This suggests that the band is contractile. Agonist-stimulated contractions or dilations may be another potential site of physiological regulation of the rectal gland and could work in concert with perfusion limitations and direct effects on cellular transport steps. However, we did not observe peristalsis in the rings (e.g. Fig. 2), and peristalsis has not been described for the rectal gland in vivo or during ex vivo perfusion. Burger (Burger, 1967) described an obvious intermittency of the gland secretion when measured in vivo, which could be the result of agonist-induced changes in gland dimensions.

In summary, our data confirm the presence of a layer of smooth muscle surrounding the glandular tissue of the elasmobranch rectal gland. In addition, rings of rectal gland from S. acanthias respond to a suite of vasoactive signalling agents, suggesting that the dimensions of the gland can be altered under the stimulus of these agents. The role of such volume changes in elasmobranch rectal gland function remains to be determined.

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