Physiology and Pharmacology of the Oviducts of Raja and Scyliorhinus

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Phil. Trans. R. Soc. Lond. B 1995 **350**, 143-151
doi: 10.1098/rstb.1995.0148

Physiology and pharmacology of the oviducts of *Raja* and *Scyliorhinus*

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SUMMARY

1. In oviparous elasmobranchs the oviducts vary in structure and physiology according to how they carry the sperm upwards and the eggs down. The sperm are accompanied by a large volume of fluid secreted by the siphon. In some species this contains much 5-hydroxytryptamine (5HT), produced by a gland. The muscles of the oviducts are very sensitive to 5HT in *Raja*, which has such a gland, but not in *Scyliorhinus*, which has none.

2. The female *Scyliorhinus* draws the egg case down the duct by attaching tendrils to a rock or weeds and swimming round and round (Dodd 1983); the muscles of the lower oviduct and vagina are weak.

3. In *Raja* the egg case is buried in the sand or mud and there are powerful muscles in the lower oviduct and vagina.

4. *In-vitro* isolated strips of longitudinal or circular muscles of the lower oviduct of *Raja* give slow rhythmical contractions. 5-HT 10^{-8} M increased the amplitude and frequency of contraction. The same response was induced by 8-OH-DPAT 5×10^{-6} M (a 5-HT$_{1A}$ receptor agonist) but not by 5×10^{-6} M 2-methyl-5-HT (a 5-HT$_3$ agonist) or by 5×10^{-6} M α-methyl-5-HT (a 5-HT$_2$ agonist). The response to 5-HT was partly blocked by methysergide 10^{-6} M (a 5-HT$_2$ antagonist) but not by ketanserin 10^{-5} M (a 5-HT$_2$ antagonist) or granisetron (BRL 43694) 10^{-5} M (a 5-HT$_3$ and 5-HT$_4$ antagonist). The receptor involved may therefore be of the 5-HT$_1$ or perhaps 5-HT$_2$ type.

5. Spontaneous contractions of the oviduct in *Raja* were inhibited by adrenalin 10^{-6} M, and less strongly by noradrenaline 10^{-6} M.

6. Substance P 10^{-8} M produced increased amplitude and frequency of contraction.

7. Acetylcholine produced contraction only above a dose of 10^{-6} M.

8. Strips of lower oviduct or vagina of *Scyliorhinus canicula* showed long periods of slight rhythmic activity punctuated by large rapid contractions at intervals of 10–40 min.

9. Similar large contractions occurred after addition of 5-HT at 10^{-6} M but only in half the experiments and at high concentration, and with long delay.

10. Adrenalin produced large rapid contractions but only at a dose of 10^{-7} M and above, and often after a long delay.

11. Substance P and acetylcholine also produced contraction, but the latter only at 10^{-6} M.

12. Strips of the oviduct or vagina of either *Raja* or *Scyliorhinus* responded to a sudden stretch by a large contraction.

13. These findings are discussed in the light of the differences in sperm transport and egg laying in the two species.

1. INTRODUCTION

Elasmobranch fishes have internal fertilization and large eggs. The oviduct therefore has important functions in carrying the sperm upwards and the eggs down. The majority of species are viviparous and in these something is known of the physiology of the ducts, especially in the placental forms (see Wourms 1977; Dodd 1983). Almost nothing is known about the movements of the oviducts in the oviparous species, although they carry the various kinds of familiar 'mermaid's purses' of dogfish, skate and ray.

Long ago it was reported (Young 1933) that stimulation of the spinal cord produced contractions of the oviduct in *Scyliorhinus*, suggesting an autonomic innervation of the reproductive tract. No other data appear to have been reported on this topic in the intervening period.

The paired oviducts are differentiated into four regions (see figure 1). The upper oviduct has thin walls and leads from a central funnel to the large oviducal, nidamental, or shell gland. This has very thick, whitish walls and a narrow lumen. It leads to the lower oviduct, which is muscular, and the two ducts then join a large median vagina opening to the cloaca. There are seven functions which these ducts must perform.

1. Reception of the claspers in the vagina. In *Scyliorhinus* there is much muscle and a thick, stratified epithelium in the adult vagina; the claspers are covered with small denticles (Leigh-Sharpe 1926).

2. Carriage of sperm upwards over long distances. Sperm are injected through the clasper accompanied

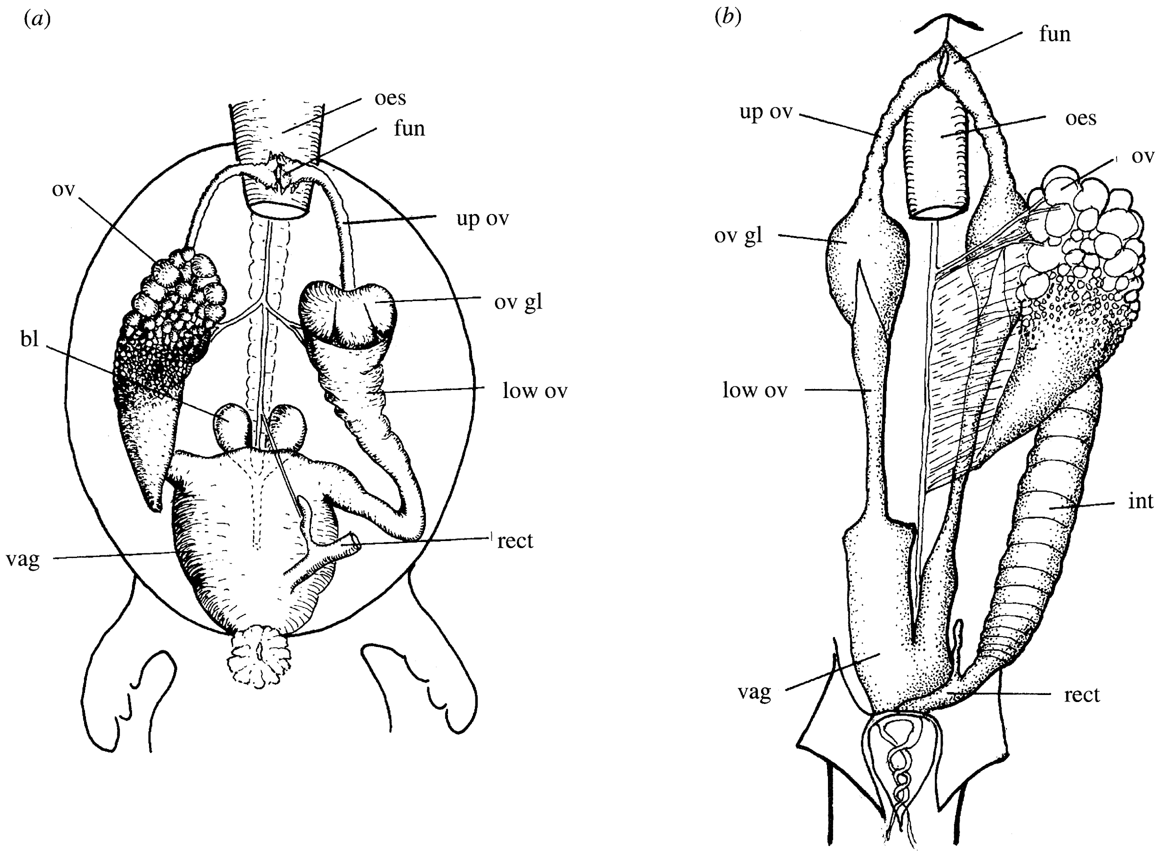

Figure 1. Diagrams of oviducts of (*a*) *Raja*, and (*b*) *Scyliorhinus*. bl, urinary bladder; fun, funnel; int, intestine; low ov, lower oviduct; oes, oesophagus; ov, ovary; ov gl, oviducal gland; rect, rectum; up ov, upper oviduct; vag, vagina.

(*in Scyliorhinus*) by a large volume of water from the siphon, which is a sac with muscular walls (Leigh-Sharpe 1920). In *Raja* the siphon is replaced by a muscular clasper gland, which produces a viscous fluid and this mixes with the sperm. In *Squalus acanthias*, which also has a gland, the liquid in the siphon contains large amounts of 5-HT (Mann 1960).

3. Storage of sperm. *S. canicula* is said to lay fertile eggs after isolation for as long as two years (Dodd 1983). Storage is presumably in the large central cavity of the oviducal gland.

4. Transport of eggs. Movement of an egg from the ovary is by an elaborate system of cilia on the peritoneal epithelium (Metten 1939) which take it to the central funnel with ciliated folds opening into the upper oviducts. These ducts have thin ciliated walls. No muscle contraction can be recorded from them (J. Young, unpublished data).

5. Formation of the egg case and its strings. The oviducal, nidamental, or shell gland – which manufactures the mermaid's purse – varies greatly according to species. That of *S. canicula* has been described in detail by Knight & Feng (1993). It is whitish or yellowish, has thick walls and a narrow lumen. It has three zones secreting albumen, mucus, and the shell and strings. The large shell-secretory region probably serves also as a receptaculum seminis. It consists mostly of a set of fine 'canniculi' whose walls secrete collagen, which is then extruded into the ducts and arranged so as to make either the plates that form the purse or the strings that are attached to the four corners. The lumen of the gland is filled with these products. There is no report of any muscles in the walls, which are wholly glandular. The nidamental gland of skates has a different form (see figure 1*a*) and produces a square purse without strings.

6. Transport of the finished purse. The lower oviduct below the shell gland is muscular with circular and longitudinal layers of muscle fibres; among these are many nerve fibres, presumably sympathetic (J. Young, personal observation). In both species this region is differentiated into a main section with thinner walls and a terminal part where the wall is very thick. The muscles presumably serve to carry down the egg case and are much thicker in *Raja* than in *Scyliorhinus*, where the egg case is pulled from the nidamental gland by strings.

7. Laying the egg. This is obviously not a function of the ducts in *Scyliorhinus*: here the two lower oviducts join in the mid-line, making a short vagina which is simply a continuation of the thickest part of the duct and opens directly to the cloaca. By contrast, in *Raja* the vagina is a very large chamber with thick tough walls (see figure 1*a*). It presumably plays an important part in burying the purse in the sand or mud on which the skate lives. Probably there are large differences in the vagina in the different species of skate. Certainly it is a powerful organ. The vagina opens to a thin-walled cloaca, which also receives the rectum and urinary ducts. There is a well-marked urinary bladder in *Raja* but not in *Scyliorhinus* (Young 1988).

This study was undertaken as part of the investi-

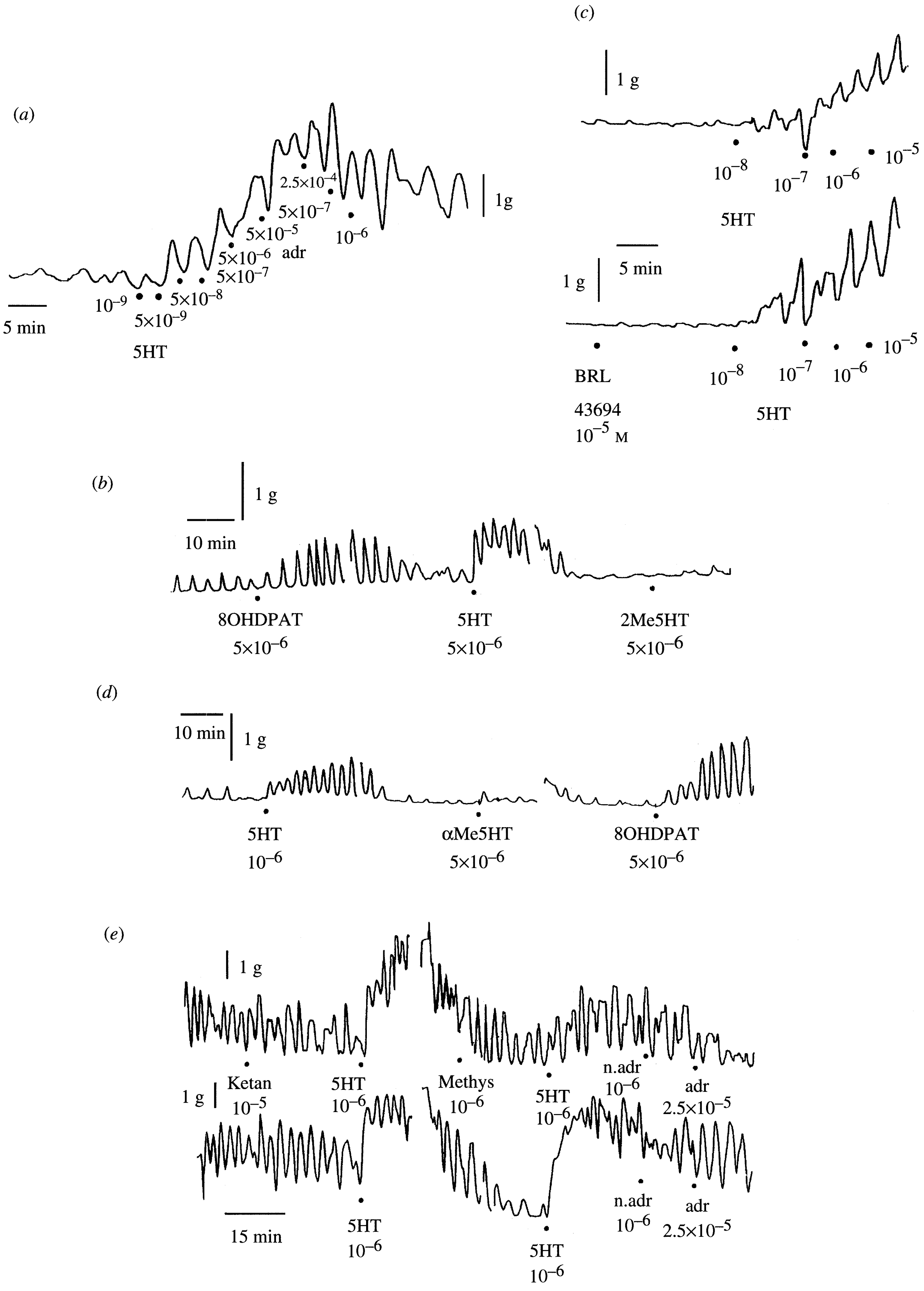

Figure 2. *Raja montagui*. Unripe female, longitudinal strips of lower oviduct. (*a*) Increasing doses of 5-HT. (*b*) Responses to increasing doses of 8-OH-DPAT (5-HT$_{1A}$ receptor agonist). No response to 2-methyl-5-HT (5-HT$_3$ receptor agonist). (*c*) Response to 5-HT not antagonized by BRL 43694 (granisetron, 5-HT$_1$ receptor antagonist). (*d*) No response to α-methyl-5-HT (5-HT$_2$ receptor agonist) but response to 8-OH-DPAT (5-HT$_1$ receptor agonist). (*e*) Two strips. Upper strip: 5-HT response not blocked by ketanserin (5-HT$_2$ receptor antagonist), reduced by methysergide (5-HT$_1$ receptor antagonist), noradrenaline produced some inhibition, adrenaline more. Lower strip: control, no ketanserin or methysergide. Noradrenaline produces inhibition, adrenaline slowing.

gation of the autonomic nervous system of elasmobranch fishes over many years (Young 1933; Andrews & Young 1993). In particular it is desirable to compare the physiology and pharmacology of the genital ducts with that of the urinary bladder (Young 1988). It is also of interest to compare these organs with those of the teleostean fishes (Young 1936) and with tetrapods (Nilsson 1983). Such information will also help towards an understanding of the functioning of these ducts.

2. MATERIALS AND METHODS

Studies were undertaken at the Marine Biological Laboratory, Plymouth, where mature female dogfish (*Scyliorhinus canicula*) were readily available. The work on skates has been largely with *Raja montagui*, the spotted ray, which becomes mature at 2–3 kg. Other species are only mature at over twice this size and are inconvenient to handle. Some experiments have been done with *R. clavata*, *R. microcellata* and *R. naevus*, mostly with nearly ripe oviducts which seem to behave like those that are fully mature.

Tissues were taken after killing by destruction of the central nervous system. Pieces of tissue were kept in chilled selachian Ringer solution composed as follows (Young 1980):

NaCl	2.7 M	108 ml
KCl	0.54 M	6 ml
CaCl	0.36 M	5 ml
$MgCl_2$	0.36 M	6 ml
Na_2SO_4	0.44 M	8 ml
$NaHCO_3$	0.54 M	11 ml
urea		27 g
distilled water to 1 l.		

Pieces 2–3 cm long were cut either across or along the ducts. They were fixed by threads to an isometric transducer so as to record either longitudinal or transverse muscles. They were immersed in a bath containing 35 ml of Ringer oxygenated by continuous bubbles of air. Washing was by emptying and refilling from below. The temperature was maintained close to that of the water in the aquarium, usually about 16 °C. The drugs were introduced by syringe from above. Those used were 5-hydroxytryptamine creatinine sulphate (5-HT), 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), Substance P, adrenaline, noradrenaline, acetylcholine, tetrodotoxin (TTX) (all Sigma), methysergide maleate (Sandoz), BRL 43694/granisetron (Smith Kline Beecham), 2-methyl-5-HT, α-methyl-5-HT (both RBI), and ketanserin (Janssen - dissolved in lactic acid).

3. RESULTS

(*a*) Raja *lower oviduct*

Isolated pieces of oviduct recorded in the longitudinal plane regularly showed slow thythmical contractions at a rate of about one every 5.4 min (32 experiments at 16 °C). The rate was similar is all nearly ripe females whether or not there was an egg case in the oviduct.

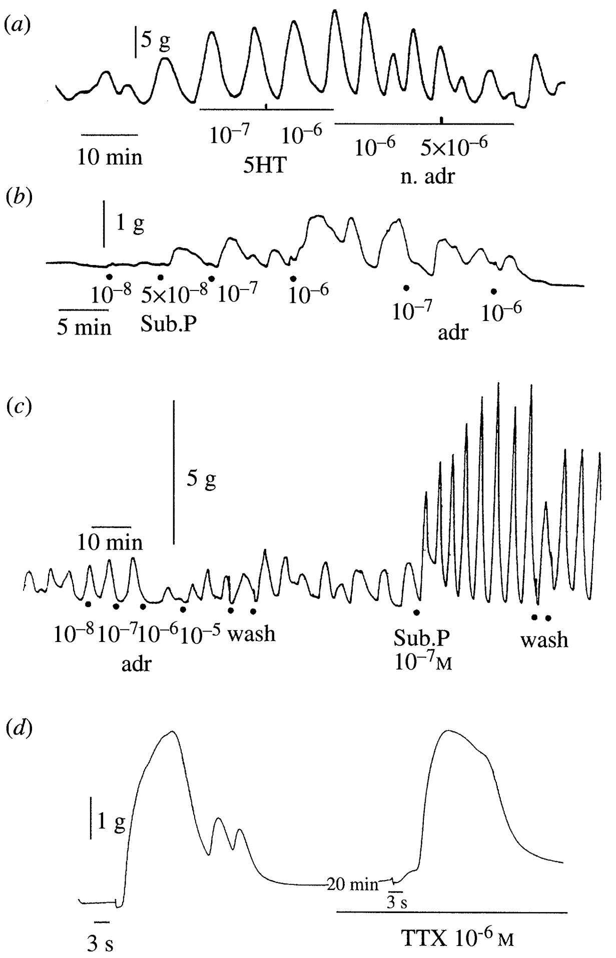

Figure 3. *Raja montagui*. Nearly ripe female. Longitudinal strips of lower oviduct. (*a*) Excitation by 5-HT, inhibition by noradrenaline. (*b*) Excitation by substance P, inhibition by adrenaline. (*c*) Inhibition by adrenaline, excitation by substance P. (*d*) Response to sharp stretch, not inhibited by TTX.

The contractions increased in amplitude and frequency after addition of 5- HT at a threshold between 10^{-9} and 10^{-8} M (see figures 2*a*,*b*,*c*). The tissues usually responded to repeated doses. The oviducts responded to 8-OH-DPAT as to 5-HT and at similar concentrations (see figure 2*b*). There was no response to 2-methyl-5-HT or α-methyl-5-HT at these concentrations (see figures 2*b*,*d*). Treatment with ketanserin (10^{-6} to 10^{-5} M) or BRL 43694 (10^{-5} M) id not block the response in five experiments (see figure 2*e*). Methysergide (10^{-6} to 10^{-5} M) reduced the amplitude of the response in six experiments (see figure 2*e*). In two cases this also sharply blocked the ongoing rhythm. Adrenaline inhibited the contractions, with reductions in the amplitude and/or frequency at concentrations of 10^{-7} upwards, and stopped them altogether at high concentrations (see figures 2*e*, 3*b*,*c*). Noradrenaline had similar but less effect (see figures 2*e* and 3*a*). Substance P produced a marked increase in amplitude and frequency at concentrations from 10^{-8} M (see figures 3*b* and *c*).

An interesting feature of the muscle of the oviduct was a rapid contraction in response to longitudinal stretch produced by tapping the thread attached to it (see figure 3*d*). There was a delay of > 2 s before the initiation of the contraction. This response occurred in

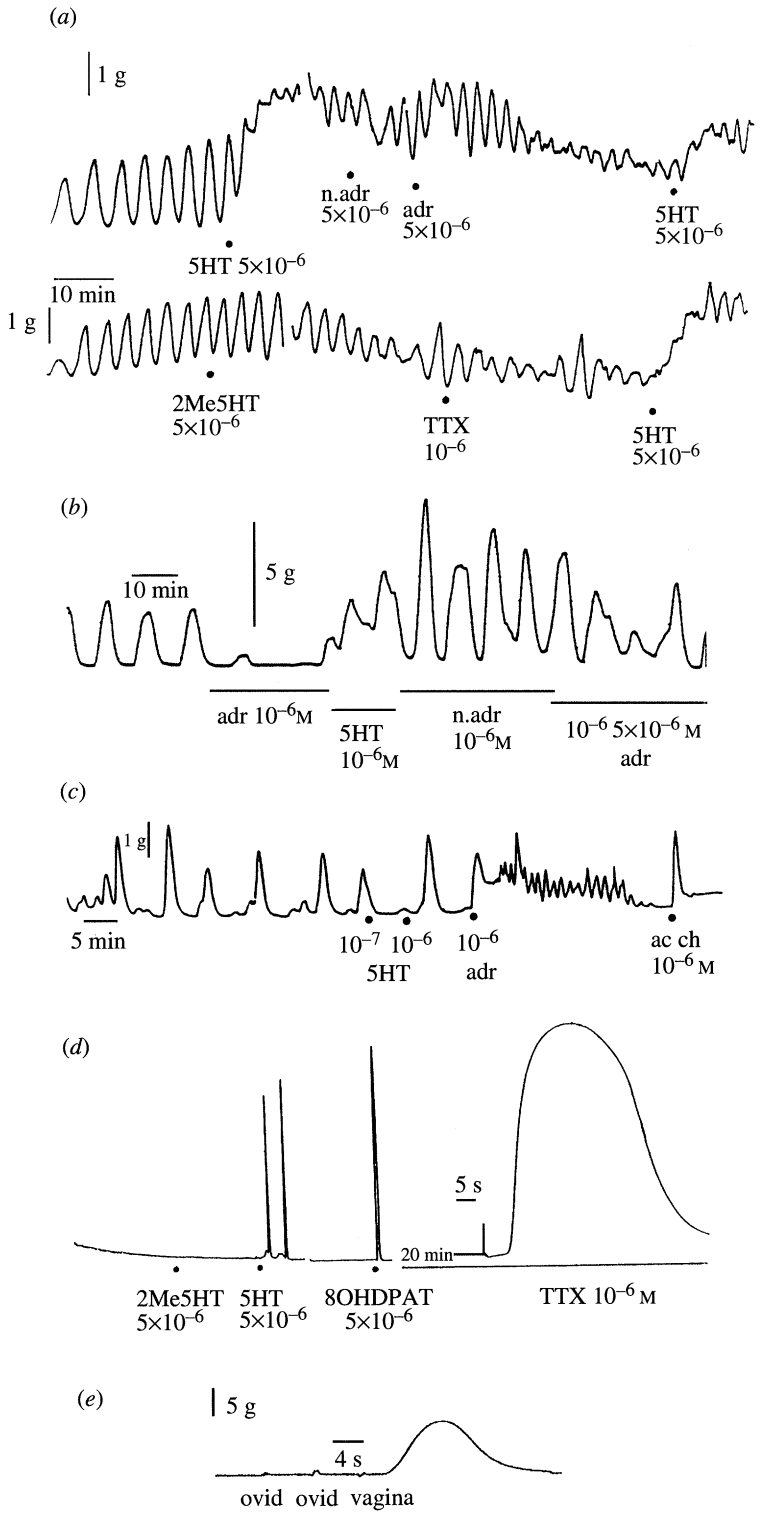

Figure 4. *Raja*. Longitudinal strips of dorsal wall of vagina. (*a*) *R. microcellata*, unripe. Upper strip: excitation by 5-HT, inhibition by adrenaline. Lower strip: no excitation by 2-me-5-HT, excitation by 5-HT in presence of TTX 10^{-6} M. (*b*) *R. montagui*, nearly ripe. Inhibition by adrenaline, less by noradrenaline. (*c*) *R. montagui*, nearly ripe. Frequent contractions with adrenaline. (*d*) *R. microcellata*, unripe. (1) Response to 5-HT and to 8-OH-DPAT but not to 2-me-5-HT. (2) Tissue pinched with forceps 25 mm away from hook, large response despite presence of TTX. (*e*) *R. microcellata*, nearly ripe. Pinching of oviduct with forceps has no effect, but large contraction on pinching vagina.

the presence of TTX 10^{-6} M, perhaps with an increased delay and amplitude (see figure 3*d*), but this has not been examined systematically.

(*b*) Raja *vagina*

The responses of longitudinal muscle from the vagina of *Raja* were in general similar to those of the lower oviduct. There are usually slow rhythmical con-

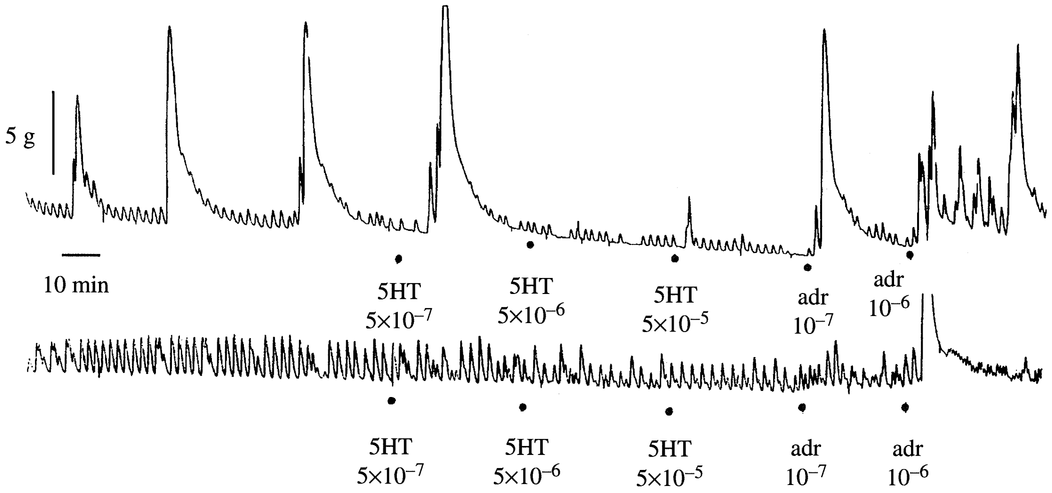

Figure 5. *Scyliorhinus*. Longitudinal strips of oviduct taken around egg case showing a delayed response to adrenaline but apparent lack of response to 5-HT.

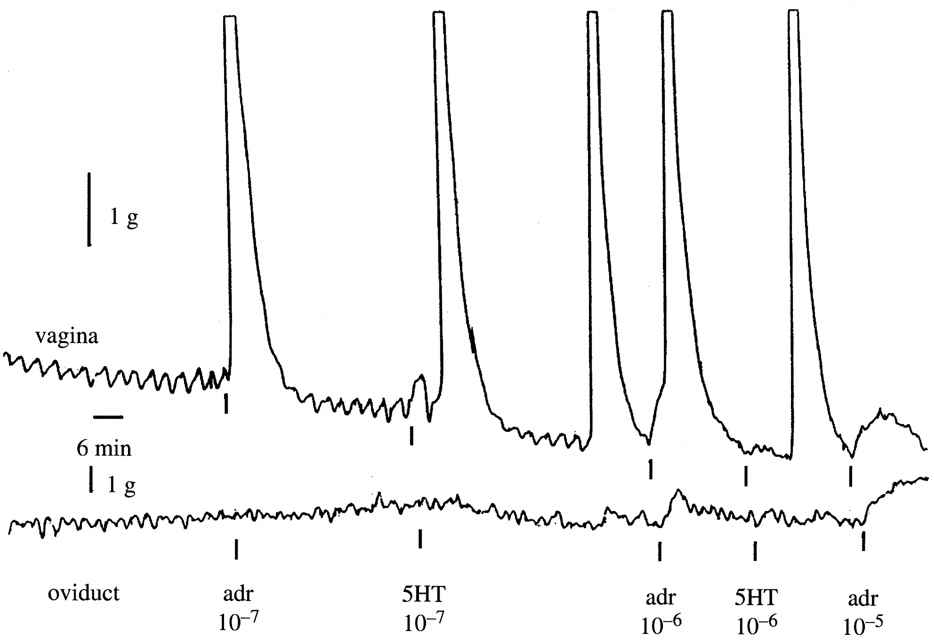

Figure 6. *Scyliorhinus*, ripe female: upper trace, strip from vagina; lower trace, longitudinal strip from oviduct showing regional differences in response to adrenaline and 5-HT.

tractions at a frequency of about one every 5 min (see figure 4*a*,*b*) but a few pieces remained inactive.

5-HT (5×10^{-6} M) and 8-OH-DPAT (5×10^{-6} M) produced large increases in amplitude and frequency, and initiated contractions if they were absent (see figure 4*a*,*d*). α-Methyl-5-HT (5×10^{-6} M) and 2-methyl-5-HT were without effect (see figure 4). The action of 5-HT continued in the presence of TTX 10^{-6} M (see figure 4*a*). Adrenaline (10^{-6} M) inhibited the contractions, whereas noradrenaline (10^{-6} M) had little effect (see figure 4*a*,*b*). At concentrations above 10^{-6} M adrenaline sometimes initiated very small amplitude and high frequency contractions (see figure 4*c*).

The vagina contracted strongly when pinched with forceps (see figure 4*e*), but this response was not given by the oviduct.

(*c*) Scyliorhinus *lower oviduct*

The ducts showed long periods of slight rhythmical activity, interspersed with occasional large rapid spontaneous contractions at variable intervals (see figure 5), which occurred whether or not there was an egg case in the duct. The small contractions occurred at a frequency of 1–2 per min. They may be of a few tenths of a gram (see figure 6), but occasionally of several grams, even with no egg case present (see figure 7).

Responses to drugs were irregular and difficult to evaluate because of the occasional spontaneous large response. Adrenaline produced contractions but only at concentrations of 10^{-7} and above (see figures 7 and 8). The responses sometimes occurred after a delay of 1 or 2 min. They were large and rapid, like the

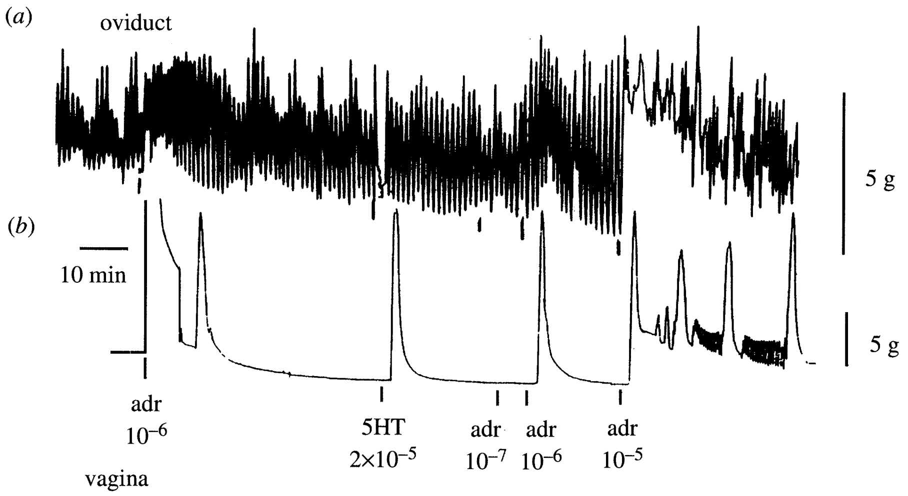

Figure 7. *Scyliorhinus*, ripe female but no eggs in oviduct: upper trace, longitudinal strip from oviduct, lower trace, longitudinal strip from vagina showing regional differences in responses to adrenaline and 5-HT.

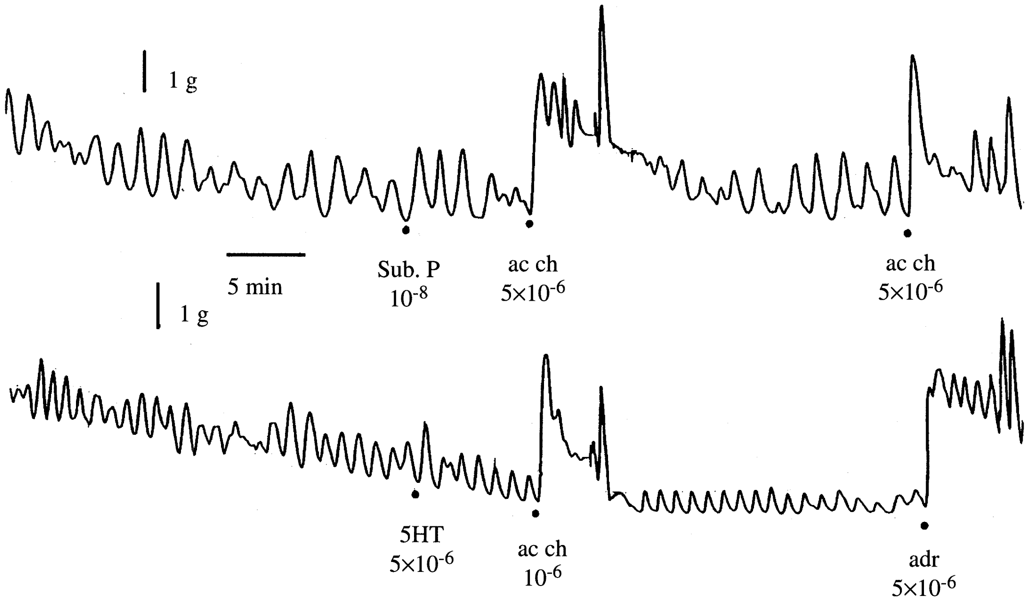

Figure 8. *Scyliorhinus*, ripe female. Two longitudinal strips from oviduct showing clear contractile responses to acetylcholine and adrenaline.

spontaneous contractions, and were sometimes followed by a series of contractions lasting for a long time (see figures 5 and 9). Contractions in response to adrenaline were seen in 22 of 24 experiments, but were sometimes slight. Responses to 5-HT were less marked than to adrenaline, and difficult to evaluate. In the main region of the oviduct, 5-HT produced responses in nine of 27 experiments and always at 10^{-6} and above (see figures 9 and 10). The response consisted of large rapid contractions, sometimes after a long delay. Responses to acetylcholine only occurred at high doses and were always large rapid contractions (see figure 8). Substance P was tested on the main duct in four experiments and in three of them contractions occurred at a concentration of 10^{-8} M (see figures 8 and 10).

Strips of oviduct responded to mechanical stimulation (stretching) with a large contraction after a long delay (see figure 11).

(*d*) **Scyliorhinus *vagina***

Tissue from the thick-walled lowest part of the oviduct and median vagina usually showed little continuous rhythmic activity. Sometimes there were small contractions at about 2 min intervals (see figure 6). There were, however, contractions which were large, rapid and spontaneous at long intervals of 30 min or more (see figures 6, 7 and 10).

There was a more regular response to adrenaline in the vagina than in the thin-walled oviduct. Contractions occurred in 23 of 28 experiments, but only at concentrations of 10^{-7} M and above. The response was always a large rapid contraction occurring after a long delay (as much as several min). At higher concentrations it was sometimes followed by a sequence of large and smaller contractions (see figure 7). There was a response to 5-HT in 15 of 29 experiments, sometimes

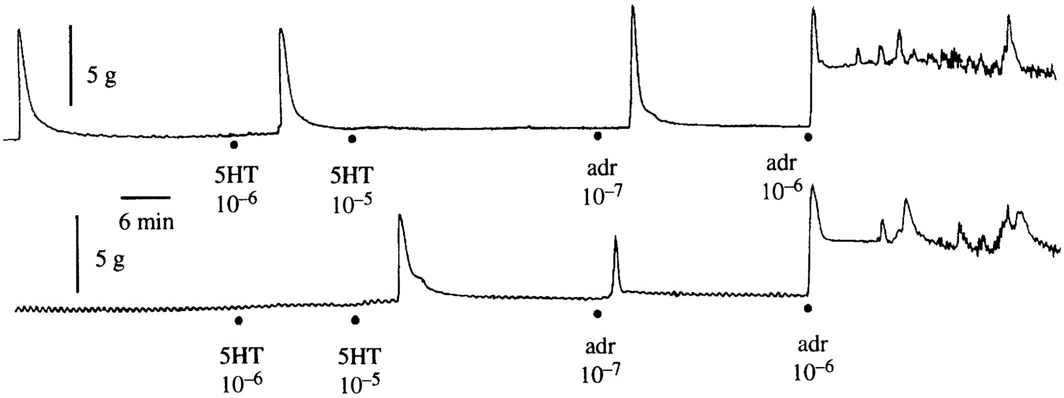

Figure 9. *Scyliorhinus*, ripe female. Two longitudinal strips from oviduct illustrating contractile responses to adrenaline and an apparent lack of response to 5-HT.

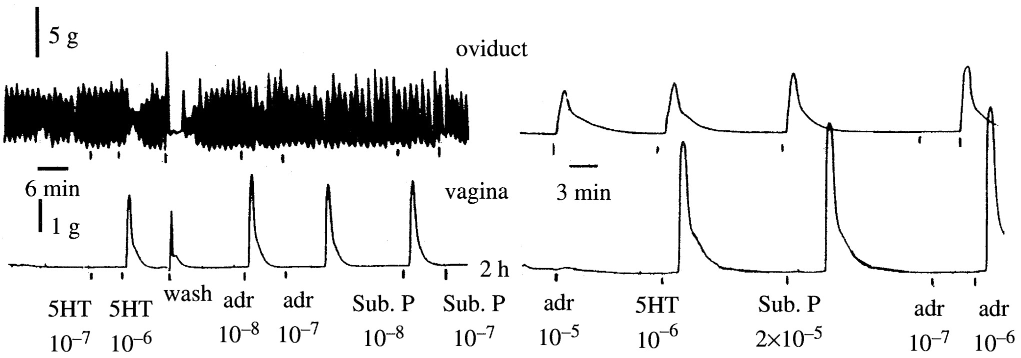

Figure 10. *Scyliorhinus*, ripe female but no eggs in oviduct. Strips from oviduct (upper trace) and vagina (lower trace) illustrating responses to 5-HT. Note the different timescales between the left and right hand panels which are separated by 2 h.

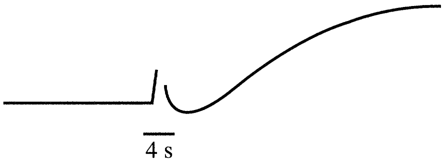

Figure 11. Scyliorhinus, ripe female but no eggs in oviduct. Longitudinal strips from oviduct, response to stretch stimulus.

at 10^{-7} M (see figure 6) but usually not below 10^{-6} M (see figure 10). The response was always a typical large rapid contraction. Occasionally a large dose of 5-HT was followed by several slow, large responses. Responses to acetylcholine only occurred at concentrations of 10^{-6} M or greater, and were rapid contractions, Substance P (10^{-8} to 10^{-5} M) produced delayed contractions in two of three experiments (see figure 10).

4. DISCUSSION

The muscles of the lower oviducts and vagina of *Raja* probably play some part in both the upward transport of sperm and the downward movement of the egg case. It is not known whether the fluid in the male siphon contains 5-HT in *Raja* as it does in *Squalus acanthias*, but the duct is very sensitive to 5-HT. The movements of the lower oviduct of *Raja* almost certainly serve to propel the egg case from the oviducal gland. The very strong muscles of the vagina seem suited to laying the egg beneath sand or mud. This action is presumably promoted by the sympathetic nerves, with 5-HT as the transmitter. Possibly substance P also plays a part, while adrenaline is inhibitory. In *Raja brachyura* the rate of egg-laying is 0.61 day^{-1} (Dodd 1983), and the eggs are laid in sand.

The response of the muscles to 5-HT is also induced by 8-OH-DPAT which is a 5-HT$_{1A}$ receptor agonist but not by 2-methyl-5-HT, a 5-HT$_3$ receptor agonist, or α-methyl-5-HT, a 5-HT$_2$ receptor agonist. Correspondingly the response to 5-HT was partly blocked by methysergide, a 5-HT$_1$ receptor antagonist, though not by ketanserin, a 5-HT$_2$ receptor antagonist or by granisetron which antagonises 5-HT$_3$ receptors. The receptor involved is therefore probably of the 5-HT$_1$ type (see Hoyer *et al* 1994 for classification of 5-HT receptors).

The functions of the ducts of *Scyliorhinus* are enigmatic. The poor response to 5-HT suggests that this transmitter is not active in sperm transport. There is no large gland in the siphon in this species and no evidence of 5-HT in the contents. The small contractions of the ducts may provide assistance to movement of the egg case by lubrication, and the large contractions presumably assist movement of the egg case.

The pharmacology of the muscles of the genital ducts of *Raja* is similar to that of the rectum and bladder, which suggests that the innervation is sympathetic (Young 1988). The muscles are made to contract by 5-HT and inhibited by adrenaline. There is no information as to whether their nerve fibres, in *Raja*, contain 5-HT. Responses to acetylcholine occurred only at high doses.

The situation is different in the teleosts *Uranoscopus* (Young 1936) and *Gadus* (Nilsson 1970). In these species the ovaries and their ducts contract in response to acetylcholine (10^{-7} M) and relax with adrenergic agents at concentrations greater than 10^{-7} M. Stimulation of the sympathetic nerves produces contraction in both species. In *Gadus*, after blocking the contraction with atropine there remains an inhibitory response to the stimulus which is blocked by propranolol and which may be adrenergic.

There is therefore some evidence that there are both excitatory and inhibitory nerves to these ducts in both elasmobranchs and teleosts, as in higher vertebrates. The excitatory fibres contain either 5-HT or acetylcholine as the transmitter, whereas the inhibition is adrenergic. There is no doubt that the situation is much more complicated than this, with other transmitters such as ATP and substance P also playing a part.

The effects of adrenaline and noradrenaline are inhibitory on these ducts in *Raja*, as they are upon the rectum and bladder of skate (Young 1988) and the urinary and genital system of teleosts. However, in *Scyliorhinus* adrenaline produces contractions in most experiments but they are often delayed or ambiguous. It appears that adrenaline is usually inhibitory to the urinary duct and bladder of fishes, as it is in most tetrapods (Nilsson 1983; Uematsu 1994).

I am very grateful to Dr P. L. R. Andrews for help in the preparation of this paper. I am also grateful to the Wellcome Trust and University College London for financial support, and to Mrs R. M. Young for secretarial assistance. The Director and Staff of the Marine Biological Association provided continuous help, and Mr J. Mavin gave valuable technical help.

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Received 10 May 1995; Accepted 28 June 1995