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HAEMODYNAMIC EFFECTS OF SECRETORY AGENTS ON THE ISOLATED ELASMOBRANCH RECTAL GLAND

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SUMMARY

Perfusion flow rate in the isolated elasmobranch rectal gland, perfused at *in vivo* pressures, was measured in *Scyliorhinus canicula* L. and *Squalus acanthias* L. Flow through the secretory parenchyma of the gland was reduced in the presence of concentrations of catecholamines in the physiological range, an effect mediated via α-adrenergic receptors within the gland vasculature. Flow through the non-secretory vascular shunts of the rectal gland was unaffected. The vasoconstriction induced by noradrenaline was blocked by the addition of cyclic AMP + theophylline or adenosine at concentrations known to stimulate secretion by the gland. In *Squalus*, a similar effect was seen with the secretagogue vasoactive intestinal peptide, but this agent had no effect in the glands of *Scyliorhinus*. Experiments indicate that the blockage of the noradrenaline effect by the secretory agents does not involve any stimulation of vasodilatory β-adrenergic receptors and, furthermore, that the vasomotor effects of these agents appear to be entirely independent of their actions on the secretory cells. Evidence is presented indicating that the vasomotor action of adenosine may be mediated via receptors specific for the ribose moiety of the nucleoside (R_a receptors) activating adenylate cyclase, and that this may, in turn, explain the observed effects of the addition of exogenous cyclic AMP. The significance of the observed vascular effects in the overall control of secretion rate by the gland *in vivo* is discussed.

INTRODUCTION

The relationship between blood flow and the secretory activity of glandular tissue has been the subject of numerous studies in the past and, in the case of mammalian salivary glands, has given rise to much controversy concerning the mechanisms involved. The salt-secreting nasal glands of birds have been studied in some detail from this viewpoint, particularly by Hanwell, Linzell & Peaker (1970, 1971), but no similar investigation has been made on the salt-secreting rectal gland of elasmobranchs. This is despite a suggestion made some 20 years ago by Burger (1962) that vasomotor changes may be a significant factor in controlling secretion rate by the

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gland. Kent, Peirce & Peirce (1973) published values of blood flow to the rectal gland in free-swimming fish, using labelled microspheres, and recorded a mean value of approximately 52 μl g gland^{-1} min^{-1} which, in the small specimens used in this study where rectal gland weight is equivalent to 0·08 % of body weight (Kent, Peirce & Bever, 1971), converts to a value of 42 μl kg^{-1} min^{-1}. Similarly, in pithed specimens of *Squalus*, Solomon *et al.* (1980) obtained a value of 3·31 ml kg^{-1} h^{-1} or 55 μl kg^{-1} min^{-1} for rectal gland blood flow in control fish.

It is known that the elasmobranch rectal gland secretes sodium and chloride ions at a concentration approximately twice that of the plasma (Burger & Hess, 1960). On appropriate stimulation, such as volume or salt loading, *in vivo* secretion rate may exceed 50 μl kg^{-1} min^{-1} (Burger, 1962), and it is clear that the above flows would be insufficient to sustain such rates, implying that blood flow to the gland must increase during secretion. This suggestion is supported by the work of Burger (1962) who, from values of secretion rate and arterial-venous differences in plasma chloride concentration, estimated rectal gland blood flow *in vivo* and obtained values of 14–114 ml h^{-1}. Recalculated on a per unit fish weight basis, this is equivalent to a range of 39–657 μl kg^{-1} min^{-1}. Small, but significant increases in rectal gland blood flow on the stimulation of secretion were also observed in pithed preparations by Solomon *et al.* (1980).

However, in the isolated perfused gland, stimulation of secretion, for example by cyclic AMP + theophylline, is not apparently accompanied by any changes in perfusion flow rate (Silva *et al.* 1980; Shuttleworth & Thompson, 1981). In view of the suggested role of circulating catecholamines in maintaining vasomotor tone in elasmobranchs (Opdyke & Holcombe, 1978), it was decided to investigate this paradox by examining the relationship between the haemodynamic effects of catecholamines and known secretory agents on the isolated perfused rectal gland.

MATERIALS AND METHODS

Most of the experiments reported here were carried out at Exeter, using the European dogfish, *Scyliorhinus canicula*, but some initial data were obtained at the Mount Desert Island Biological Laboratory (MDIBL), Maine, U.S.A., using specimens of *Squalus acanthias*, and have been reported in a preliminary form elsewhere (Shuttleworth & Thompson, 1981). The results obtained with the two species were qualitatively and quantitatively similar, with the notable exception of the effect of vasoactive intestinal peptide (see Results).

Specimens of *Scyliorhinus* weighing 0·7–1·1 kg were obtained from the Plymouth Laboratory of the Marine Biological Association and were kept in large fibreglass tanks, supplied with sea water at a temperature of 11 °C, until use. Fish were pithed, opened ventrally, and most of the intestine removed to a point approximately 3 cm anterior to the rectal gland. Glands were perfused *in situ* under constant pressure conditions via a cannula inserted into the single rectal gland artery (modified posterior mesenteric artery). Perfusion flow through the gland was determined by cannulating the rectal gland vein that exits from the posterior end of the gland and immediately reflects anteriorly along the dorsal surface of the gut. Kent & Olson (1978) have shown that flow in this vein represents that which has perfused the secretory parenchyma of the gland, as opposed to various non-secretory shunt pathways that generally drain

Table 1. *Composition of the dogfish saline used* ($mmol\,l^{-1}$)

Sodium chloride	257	Magnesium sulphate	3
Sodium sulphate	8	Disodium hydrogen phosphate	0·1
Sodium hydrogen carbonate	6	Urea	400
Potassium chloride	4	Trimethylamine oxide	40
Calcium chloride	2		

Glucose ($30\,mmol\,l^{-1}$) was added immediately before use. pH = 7·7 when equilibrated with 0·2% CO_2 in oxygen.

small diffuse vessels in the wall of the intestine. For the measurement of flow, the efferent venous cannula was led to a direct reading rotameter-type flowmeter (Gilmont). Occasionally an additional flowmeter was inserted into the afferent line, enabling a comparison to be made between the total afferent flow and efferent flow via the rectal gland vein. By subtraction, it was therefore possible to calculate the proportion of the total perfusion flow exiting via the non-secretory vascular shunts.

Afferent perfusion pressure was maintained at 17 mmHg, approximating dorsal aorta blood pressures in this species (Butler & Taylor, 1975), by means of a pump/overflow system supplied from a reservoir of saline (Table 1) gassed with 0·2% carbon dioxide in oxygen. Efferent venous pressure was maintained at zero with respect to the gland. All experiments were carried out in a constant temperature room at 11 °C. Drugs were added to the saline reservoir in amounts appropriate to give the final desired concentration in the perfusate.

In the preliminary work carried out at MDIBL, specimens of *Squalus* weighing 3–7 kg were caught in Frenchman's Bay, Maine, and kept in live cars until use. The technique used was essentially the same as that described above for *Scyliorhinus*, except that the glands were completely removed from the fish and perfused, in isolation, at a pressure of 20 mmHg with saline gassed with 1 % carbon dioxide in oxygen. The preparation was maintained at a temperature of 13–15 °C by placing it on a thin Perspex plate which formed the roof of a water-cooled chamber. In addition, it is possible to measure secretion rate in the very much larger glands of *Squalus* (approximately 1·0–2·7 g wet weight as compared with 0·06–0·12 g wet weight in *Scyliorhinus*). The secretory ducts of isolated perfused *Squalus* glands were cannulated via their opening on the mucosal surface of the posterior intestine using polyethylene tubing. This cannula was led to a second saline-filled direct reading rotameter flowmeter (Gilmont) for the determination of secretory flow rate.

Drugs used were noradrenaline bitartrate (L-arterenol bitartrate), isoprenaline bitartrate (isoproterenol bitartrate), adenosine and dibutyryl cyclic AMP, (all from Sigma), phentolamine mesylate (CIBA), propranolol hydrochloride (Inderal, ICI), theophylline (BDH), vasoactive intestinal peptide (synthetic porcine, Peninsular Laboratories), and furosemide (generous gift from Hoescht Pharmaceuticals).

RESULTS AND DISCUSSION

Under the conditions used in this study, efferent perfusion flow, as measured in *Scyliorhinus* glands, was $8{\cdot}13 \pm 0{\cdot}41\,ml\,g^{-1}\,min^{-1}$ (mean ± S.E., $N = 25$). This

constituted 64·6 ± 1·7 % of the total afferent flow (mean ± S.E., $N = 5$), indicating that, under control conditions, approximately one-third of the total afferent flow to the gland passed through non-secretory vascular shunts. Despite a very much lower average efferent perfusion flow in isolated *Squalus* glands of 1·72 ± 0·14 ml g^{-1} min^{-1} (mean ± S.E., $N = 17$), this again accounted for approximately two-thirds (70·0 ± 2·1 %, $N = 17$) of the total afferent flow, as in *Scyliorhinus*. The weight of the gland in *Scyliorhinus* is approximately 0·1 g per kg fish, whilst that in the *Squalus* used was approximately 0·4–0·5 g per kg fish. When these values are taken into account, the rectal gland flow *per weight of fish* is very similar in the two species (i.e. approximately 0·8 ml kg^{-1} min^{-1}). The precise significance of this, if any, is not clear, but the similarity, despite the pronounced differences in the weights of the specimens used and the relative sizes of the glands between the species, is certainly of some interest. It is also apparent that this rate of flow is considerably higher than the values of blood flow to the gland *in vivo* reported by Kent *et al.* (1973) and Solomon *et al.* (1980). The fact that it is similar to the *maximum* value of blood flow calculated from the data of Burger (1962) of 0·657 ml kg^{-1} min^{-1} (see above) suggests that the isolated gland perfused *in vitro* may be in a maximally vasodilated state.

Effect of catecholamines

Fig. 1 shows the effect of various concentrations of noradrenaline on efferent perfusion flow through the gland of *Scyliorhinus*. At concentrations above 10^{-8} M, this catecholamine produces a pronounced reduction in perfusion flow indicating an induced increase in vascular resistance within the gland. Statistical analysis of the concentration-response curve indicates a value for the concentration of noradrenaline

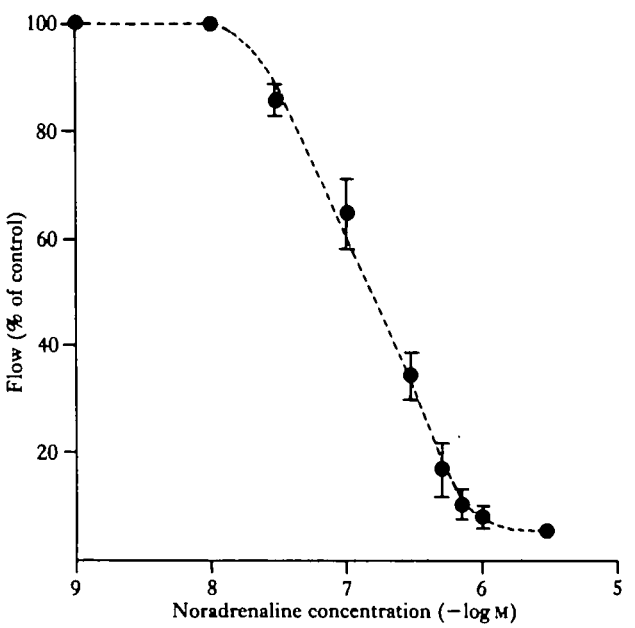

Fig. 1. Concentration response curve for noradrenaline on efferent perfusion flow. All values expressed as a percentage of original flow in absence of noradrenaline. $\bar{x} \pm$ S.E. ($N = 5$) in each case.

ving a half-maximal response (ED_{50}) of $1{\cdot}3 \times 10^{-7}$ M. Unlike noradrenaline, the specific β-adrenergic agonist isoprenaline, even at concentrations as high as 10^{-6} M, was totally without effect on the perfusion flow (Fig. 2). The α-adrenergic antagonist phentolamine, at a concentration of 10^{-6} M, completely reverses the reduction in perfusion flow induced by noradrenaline (Fig. 2), whilst the β-adrenergic antagonist propranolol was without effect (not shown). The data presented in Table 2 show that noradrenaline specifically reduces that proportion of the flow passing through the secretory parenchyma of the gland itself and that the flow through the non-secretory shunts is completely unaffected by the catecholamine.

Normal resting levels of catecholamines in the plasma of *Scyliorhinus* have been measured by Butler, Taylor, Capra & Davison (1978), who found mean values of 32·1 pmol ml^{-1} ($3{\cdot}2 \times 10^{-8}$ M) for noradrenaline and 25·6 pmol ml^{-1} ($2{\cdot}56 \times 10^{-8}$ M) for adrenaline. It can be seen, therefore, that perfusion flow through the rectal gland is sensitive to noradrenaline at concentrations within the physiological range, and this

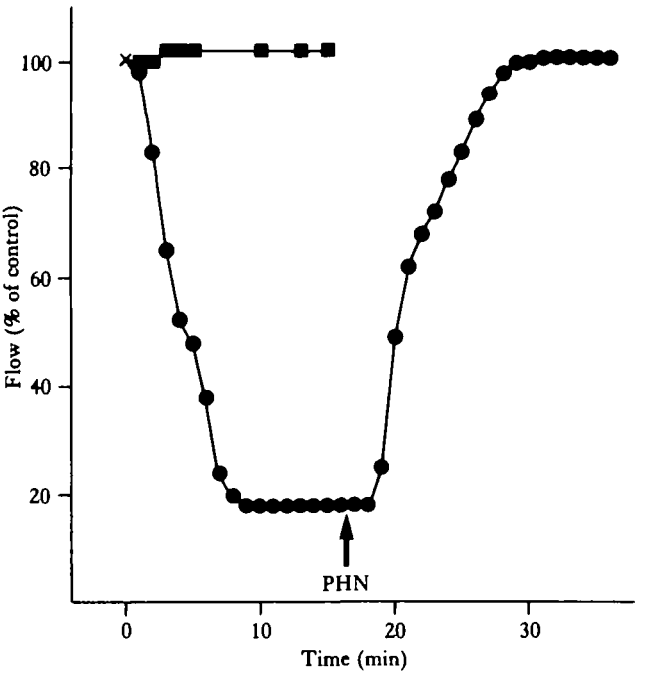

Fig. 2. Effect of isoprenaline and phentolamine on efferent perfusion flow. Typical results illustrated. At time zero (X), glands were perfused with saline containing 10^{-6} M-isoprenaline (■), or 5×10^{-7} M-noradrenaline (●). At the point indicated, 10^{-6} M-phentolamine (PHN) was added.

Table 2. *Effect of noradrenaline (5×10^{-7} M) on perfusion flow in the rectal gland*

	Total afferent flow	'Gland flow'	'Shunt flow'
Control (C)	13·23 ± 1·33	8·55 ± 1·10	4·67 ± 0·29
+ Noradrenaline (NA)	6·15 ± 0·64	1·35 ± 0·22	4·80 ± 0·52
Difference (NA − C)	−7·08 ± 0·81	−7·21 ± 1·00	+0·13 ± 0·23

Flows in ml g^{-1} min^{-1}, $\bar{x}$ ± S.E. $N = 4$ in all cases.

would suggest that blood supply to the gland *in vivo* may well be restricted under the influence of circulating levels of catecholamines. In this context, it may be of some significance that perfusion flow in the *maximally* constricted isolated gland is some 5% of the original flow. For *Squalus*, this would represent a flow of 86 μl g^{-1} min^{-1} or 35–45 μl kg fish^{-1} min^{-1}, a value very close to those recorded in the *in vivo* studies of Kent *et al.* (1973) and Solomon *et al.* (1980). This suggests that the fish used by these workers may well have been experiencing high levels of circulating catecholamines, probably produced as a result of experimental stress following cannulation or pithing. Various stresses are known to increase plasma catecholamines in other fish species (Nakano & Tomlinson, 1967), and Butler *et al.* (1978) showed that a hypoxic stress induced greater than ten-fold increases in noradrenaline and adrenaline concentrations in the plasma of *Scyliorhinus*. It can be seen from Fig. 1 that such concentrations would produce an almost maximum vasoconstriction within the rectal gland.

Effect of secretory agents

As previously reported for *Squalus* (Silva *et al.* 1980; Shuttleworth & Thompson, 1981), addition of cyclic AMP (cAMP) and theophylline, at concentrations known to produce a pronounced stimulation of secretion, failed to alter perfusion flow in the isolated glands of *Scyliorhinus*. However, the addition of 5×10^{-5} M-cAMP and $2{\cdot}5 \times 10^{-4}$ M-theophylline to glands perfused with noradrenaline reverses the vasoconstriction induced by the catecholamine (Fig. 3). Similarly, the addition of 10^{-5} M-adenosine, which also produces a large increase in secretion in the isolated glands of *Squalus*, reverses the vasoconstriction seen in glands perfused with noradrenaline (Fig. 3). In both cases, the reversal of the noradrenaline-induced vasoconstriction is both rapid and virtually complete at the concentrations used and occurs despite the continued presence of the catecholamine in the perfusate. As with the results of noradrenaline alone, the addition of cAMP + theophylline had no effect on the perfusion flow through the non-secretory shunt pathways.

Vasoactive intestinal peptide (VIP) is also a potent secretory agent in the isolated glands of *Squalus* (Stoff *et al.* 1979), and in this species low concentrations (2×10^{-8} M) of the hormone produced similar effects to those found with cAMP + theophylline and adenosine (Fig. 4). However, it was found that VIP was completely without effect, under identical circumstances, in the isolated gland of *Scyliorhinus*, even at concentrations as high as 10^{-6} M. The reason for this discrepancy is not clear, but it may be of some significance that VIP also fails to produce any increases in ouabain binding and oxygen consumption in slices of *Scyliorhinus* glands (T. J. Shuttleworth, unpublished data). Both of these parameters are associated with increases in secretory activity induced by cAMP + theophylline (Shuttleworth & Thompson, 1980*a*), and the failure of VIP to affect the rectal gland in *Scyliorhinus* compared to the clear effects at low concentrations in *Squalus* appears to represent a pronounced and, as yet, unexplained species difference.

Fig. 5 shows full concentration-response curves for noradrenaline in the presence of either cAMP + theophylline or adenosine in the isolated glands of *Scyliorhinus*. In the presence of the secretory agents, the concentration-response curves are shifted to the right, producing values of the ED_{50} for noradrenaline of $1{\cdot}3 \times 10^{-6}$ M in the

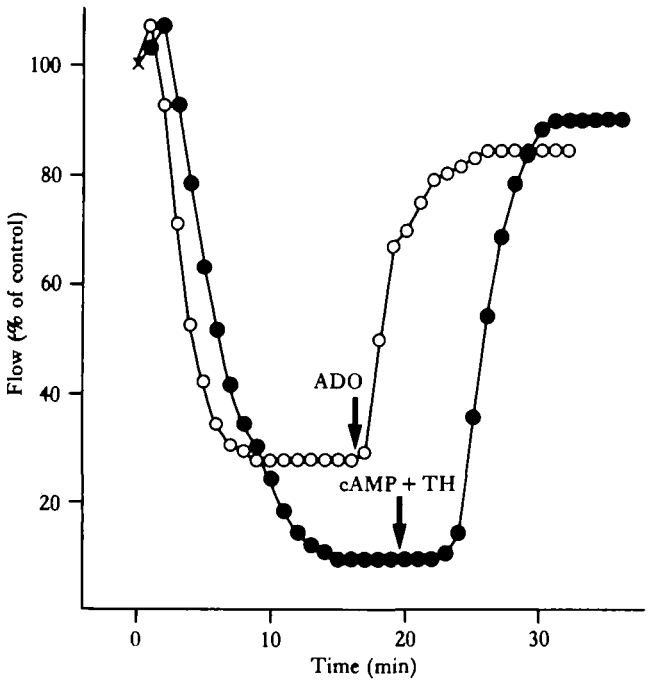

Fig. 3. Effect of secretory agents on efferent perfusion flow in the presence of noradrenaline. Typical results illustrated. At time zero (X), glands were perfused with saline containing either 3×10^{-7} M (○) or 5×10^{-7} M (●) noradrenaline. At the points indicated, 10^{-5} M-adenosine (ADO) or 5×10^{-5} M-cAMP + $2{\cdot}5 \times 10^{-4}$ M-theophylline (cAMP + TH) were added.

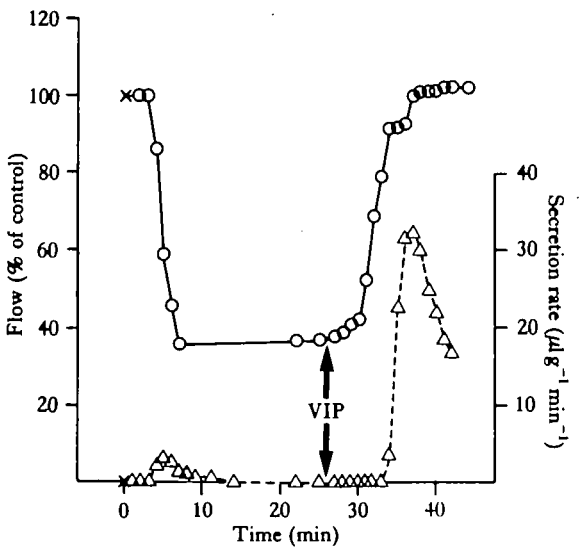

Fig. 4. Effect of vasoactive intestinal peptide (VIP) on efferent perfusion flow (○) and secretion rate (△) in *Squalus acanthias*. Typical result illustrated. At time zero (X), gland was perfused with saline containing 3×10^{-7} M-noradrenaline. At the point indicated, 2×10^{-8} M-VIP was added.

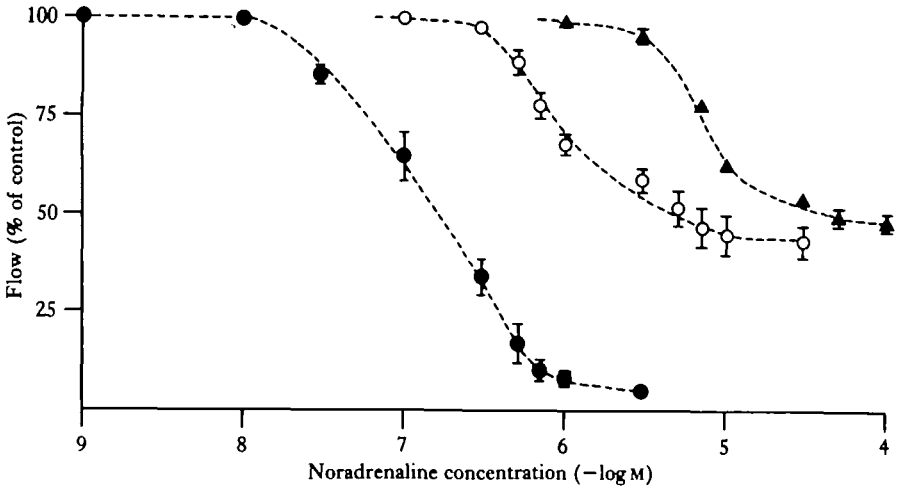

Fig. 5. Concentration response curves for noradrenaline on efferent perfusion flow in the presence of secretory agents. All values expressed as percentage of original flow in absence of noradrenaline. ● control; ○ + adenosine (10^{-5} M); ▲ + cAMP (5×10^{-5} M) and theophylline ($2{\cdot}5 \times 10^{-4}$ M). $\bar{x} \pm$ S.E. ($N = 5$) in each case.

presence of adenosine, and $7{\cdot}8 \times 10^{-6}$ M in the presence of cAMP + theophylline. In addition, it can be seen that, with the secretory agents, the *maximum* effect of noradrenaline, even when used in concentrations as high as 10^{-4} M, is only approximately 50 % of that seen in the absence of the secretory agents. The β-adrenergic agonist isoprenaline does not elicit any reversal of the noradrenaline-induced vasoconstriction (Fig. 6) and, furthermore, the normal response to adenosine is not blocked by the β-adrenergic antagonist propranolol (Fig. 6). This indicates that the effective vasodilations induced by the secretory agents do not involve any stimulation of β-adrenergic receptors which, in fact, do not appear to be present in the vasculature of the rectal gland.

Relationship between secretory and vasomotor effects

It is known that the stimulation of secretion is associated with large increases in oxygen consumption by the gland cells (Shuttleworth & Thompson, 1980*b*; Silva *et al.* 1980). The possibility exists, therefore, that the observed increases in perfusion flow induced by the addition of the adenosine and cAMP + theophylline to glands perfused with noradrenaline may simply be the result of a vasodilation caused by hypoxia subsequent to the stimulation of secretory activity. However, reduction of the P_{O_2} of the perfusate to 20 % of its original level, by gassing with air instead of 0·2 % carbon dioxide in oxygen, failed to have any effect on rate of perfusion flow in the presence of 5×10^{-7} M-noradrenaline. This hypothesis was further investigated by studying the effect of the diuretic furosemide on the vascular effects of the secretory agents, and the results are presented in Table 3. Furosemide, at a concentration known to inhibit secretory activity in the gland (Silva *et al.* 1977; Shuttleworth & Thompson, 1980*a*), had no effect on the vasoconstriction induced by noradrenaline. More significantly, the diuretic was completely without effect on the inhibition of the noradrenaline-induced reduction in perfusion flow observed in the presence of t

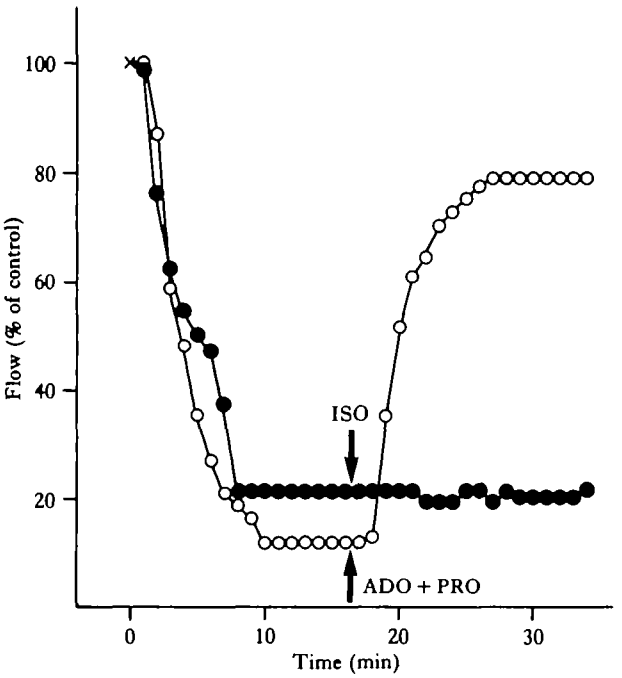

Fig. 6. Effect of beta-adrenergic stimulation and inhibition on efferent perfusion flow. Typical results illustrated. At time zero (X), glands were perfused with saline containing either 5×10^{-7} M (●) or 3×10^{-7} M (○) noradrenaline. At the points indicated, 10^{-6} M-isoprenaline (ISO) or 10^{-5} M-adenosine + 10^{-6} M-propranolol (ADO + PRO) were added.

Table 3. *Effect of furosemide (5×10^{-4} M) and secretory agents on efferent perfusion flow in rectal glands perfused with noradrenaline*

	Noradrenaline (3×10^{-7} M)	
Secretory agent	Control	+ Furosemide
None	34·0 ± 4·50 (5)	29·2 ± 2·94 (4)*
Adenosine	97·8 ± 1·25 (5)	94·4 ± 5·10 (4)*
cAMP/theophylline	101·2 ± 1·46 (4)	100·0 ± 0 (4)*

All values are expressed as a percentage of the original flow without noradrenaline. Concentrations of secretory agents used were: adenosine, 10^{-5} M; cAMP, 5×10^{-5} M; theophylline, $2{\cdot}5 \times 10^{-4}$ M. $\bar{x} \pm$ S.E. (N) in all cases.
* Not significantly different from the corresponding control value.

secretory agents adenosine and cAMP + theophylline. Furosemide is believed to have its effect by blocking the coupled uptake of sodium and chloride ions across the basolateral membranes of the secretory cells. As such, furosemide inhibits not only secretion but also the increases in secretion-related parameters such as ouabain-sensitive oxygen consumption, ouabain binding, and sodium and chloride uptake into the secretory cells that are seen on the addition of cAMP + theophylline (Shuttleworth & Thompson, 1980*a*). The fact that the inhibition of secretion and the above secretion-related phenomena was completely without effect on the vasomotor effects of the secretory agents clearly suggests that the vascular and secretory actions these agents are entirely independent of each other.

Nature of the vasomotor effects

Adenosine could be exerting its effects on the rectal gland vasculature either intracellularly or extracellularly via specific receptors associated with stimulatory or inhibitory actions on membrane-bound adenylate cyclase (Londos, Cooper & Wolff, 1980). It is known that low concentrations of methylxanthines, such as theophylline, are capable of blocking receptors specific for the ribose moiety of the adenosine molecule (Smellie, Davis, Daly & Wells, 1979). The results in Table 4 show that the observed action of adenosine in blocking the vasoconstriction induced by noradrenaline is itself inhibited by the simultaneous presence of 10^{-5} M theophylline. This suggests that ribose-specific stimulatory adenosine receptors (R_a receptors) are present on the vascular elements in the gland and that they are responsible for the mediation of the vasomotor effects of the nucleoside. It has been suggested that similar receptors exist on the secretory cells of the rectal glands of *Squalus* (Erlij, Silva & Rubio, 1980; Forrest, Rieck & Murdaugh, 1980). According to the classification of Londos *et al.* (1980), such receptors produce their effects by activating adenylate cyclase and increasing intracellular levels of cAMP. This is supported by the similarity seen in the rectal gland between the vasomotor effects of adenosine and the addition of exogenous cAMP together with theophylline, which at a concentration of $2{\cdot}5 \times 10^{-4}$ M would act as an inhibitor of phosphodiesterase activity (Smellie *et al.* 1979). It is known that β-adrenergic-mediated relaxation of smooth muscle frequently involves activation of adenylate cyclase and increases in intracellular cAMP levels (Bär, 1974). Despite the apparent absence of β-adrenergic receptors in the vasculature of the rectal gland, a vasodilatory (i.e. relaxing) mechanism involving cAMP is suggested which may, in this tissue, be activated by adenosine receptors and possibly by other receptors for physiological effectors that are, as yet, unidentified.

To summarize, in the elasmobranch rectal gland, low levels of catecholamines, acting via α-adrenergic receptors, produce a pronounced vasoconstriction reducing flow through the secretory parenchyma of the gland. As this effect occurs within the physiological range of noradrenaline concentrations in the plasma, it is likely that blood flow through the gland is normally restricted *in vivo* by circulating catecholamines. This vasoconstriction is, however, abolished in the presence of agents known to stimulate secretion by the gland, effectively shifting the normal noradrenaline concentration-response curve so that essentially all significant catecholamine effects are blocked at physiological concentrations. Clearly such action could be the basis of an increase in blood flow to the gland associated with the

Table 4. *Effect of equimolar theophylline on the normal adenosine response on efferent perfusion flow in the rectal gland*

	N	Control	+ Noradrenaline (3×10^{-7} M)
Saline	5	$8{\cdot}29 \pm 0{\cdot}68$	$2{\cdot}80 \pm 0{\cdot}61$*
Adenosine	5	$6{\cdot}74 \pm 1{\cdot}02$	$6{\cdot}58 \pm 1{\cdot}00$
Adenosine + theophylline	4	$5{\cdot}99 \pm 0{\cdot}48$	$2{\cdot}34 \pm 0{\cdot}23$*

All flows in $ml\,g^{-1}\ min^{-1}$, $\bar{x} \pm$ S.E.. Concentrations used: adenosine, 10^{-5} M; theophylline, 10^{-5} M.
* Significantly different from control value ($P < 0{\cdot}001$).

stimulation of secretion *in vivo* as observed by Solomon *et al.* (1980). The evidence suggests that the observed vasomotor activities of the secretory agents are independent of their action on the secretory cells and are mediated via direct effects on vascular smooth muscle in the gland, probably involving changes in intracellular cAMP levels. Nevertheless, it seems likely that the vascular effects of the secretory agents would form an important and integral part of the overall physiological control of secretion rate by the gland *in vivo*.

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