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α_2-Adrenoceptor-mediated contractile response to catecholamines in smooth muscle strips isolated from rainbow trout stomach (*Salmo gairdneri*)

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1 The type of adrenoceptor involved in the contractile response to catecholamines in smooth muscle strips isolated from rainbow trout stomach was determined.

2 Noradrenaline (10 nM–10 μM) and adrenaline (10 nM–3 μM) caused non-sustained contractions which were markedly decreased by phentolamine (5.4 μM) but not by carteolol (5 μM). Phenylephrine (1 μM–1 mM) was less effective in causing muscle contraction and methoxamine produced no contraction. Clonidine (100 nM–300 μM) caused no mechanical response but inhibited the contraction to noradrenaline or adrenaline but not acetylcholine or 5-hydroxytryptamine.

3 Yohimbine (10 nM–1 μM) decreased the contraction induced by noradrenaline or adrenaline but prazosin (1 μM) did not.

4 Tetrodotoxin (780 nM) partially reduced the contraction induced by noradrenaline or adrenaline but atropine (500 nM) did not.

5 In the presence of atropine (1 μM), electrical transmural stimulation caused frequency-dependent, tetrodotoxin-sensitive contractions.

6 These results suggest that the contractile response induced by noradrenaline or adrenaline is mediated by α_2-adrenoceptors. It is also suggested that noradrenaline and adrenaline contract the smooth muscle by direct action and by indirect action through the non-cholinergic excitatory nerve.

Introduction

Three subclasses of adrenoceptors have been postulated for the gastrointestinal smooth muscle of mammals. They are α_1-, α_2- and β-adrenoceptors: α_1- and β-adrenoceptors are present on the smooth muscle cells. Activation of β-adrenoceptors causes relaxation and that of α_1-adrenoceptors causes relaxation or contraction of the intestinal smooth muscle (Bowman & Hall, 1970; Wikberg, 1977; 1978; 1981; Bauer, 1981; Sahyoun *et al.*, 1982). On the other hand, α_2-adrenoceptors are present on both enteric cholinergic nerve terminals and smooth muscle cells. α_2-Adrenoceptors on the prejunctional site regulate cholinergic neurotransmission in an inhibitory manner (Gillespie & Khoyi, 1977; Wikberg, 1977; Drew, 1978; Bauer, 1981; Nakahata *et al.*, 1982) and those on smooth muscle cells (postjunctional site) participate in smooth muscle contraction (Sahyoun *et al.*, 1982) or smooth muscle relaxation (Bauer, 1982; Bauer & Kuriyama, 1982).

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Noradrenaline or adrenaline cause contraction of smooth muscle of the gastrointestinal tract of some species of teleost fish (Burnstock, 1958; Nilsson & Fänge, 1967; 1969; Edwards, 1972; Campbell & Gannon, 1976). Electrical stimulation of the vagus nerve of the cod (Nilsson & Fänge, 1969) or of the splanchnic nerve of the rainbow trout (Campbell & Gannon, 1976) causes contraction of gastric smooth muscle, an effect which is inhibited by the adrenergic neurone blocking agent, bretylium. These observations indicate that adrenergic stimulation causes an excitatory response in the gastrointestinal smooth muscle of teleost fish. Nilsson & Fänge (1969) reported that, since the contractile response of longitudinal smooth muscle of the cod stomach induced by catecholamines or vagus nerve stimulation was inhibited by phenoxybenzamine, and since isoprenaline was not effective in causing contraction, α-adrenoceptors mediated the contraction. However, there have been few reports concerning the adrenoceptor types in the gastrointestinal tract of teleost fish. Therefore, the

receptor mechanisms involved in the excitatory response to catecholamines have not been elucidated.

In the present experiments, we selected longitudinal muscle strips of the rainbow trout stomach because of their responsiveness to the contractile effect of noradrenaline or adrenaline, and examined the type of adrenoceptor and the mechanism of the response by use of selective adrenoceptor agonists and antagonists.

Methods

A male or female rainbow trout (*Salmo gairdneri*) (100 g–200 g in body weight) was stunned by a blow on the head and then bled. The abdominal cavity was opened and the stomach was removed. Longitudinal muscle strips (about 30 mm long and 2 mm wide) from the cardiac region of the rainbow trout stomach were prepared and suspended in an organ bath (8 ml) containing a modified Krebs solution of the following composition (millimolar concentrations): NaCl 100.8, KCl 3.35, $CaCl_2$ 2.52, KH_2PO_4 1.11, $MgSO_4$ 1.17, $NaHCO_3$ 25 and glucose, 11.1. The organ bath solutions were maintained at 20 ± 1°C and equilibrated with 95% O_2 plus 5% CO_2 (pH 7.4). Each stomach strip was connected to a force displacement transducer (Nihon Kohden, TB-112T) that was coupled to an ink-writing polygraph to record the isometric contractions. The preparations were loaded initially with 2 g tension and allowed to equilibrate for approximately 1 h. During the equilibration period, the tonus of the preparations decreased gradually to a steady level (0.5 g–0.7 g).

Neuronal components in the wall of the stomach were stimulated electrically, through two platinum ring electrodes placed at the top and the bottom of the organ bath. A pulse generator (Nihon Kohden, SEN-3201) was used to deliver rectangular pulses (supramaximal voltage, 1 ms duration) for 10 s at 5 min intervals.

The following drugs were used; acetylcholine chloride (Wako), adrenaline bitartrate (Tokyo Kasei), atropine sulphate (Tokyo Kasei), carteolol hydrochloride (Otsuka), clonidine hydrochloride (Tokyo Kasei), hexamethonium chloride (Wako), 5-hydroxytryptamine creatinine sulphate complex (Sigma), isoprenaline hydrochloride (Tokyo Kasei), methoxamine hydrochloride (Nihon Shinyaku), noradrenaline bitartrate (Wako), papaverine hydrochloride (Wako), phentolamine mesylate (Ciba), phenylephrine hydrochloride (Tokyo Kasei), prazosin hydrochloride (Tokyo Kasei), tetrodotoxin (Sankyo) and yohimbine hydrochloride (Tokyo Kasei). All drugs were dissolved in distilled water immediately before use and applied in the organ bath by micropipette.

Student's t test was used to determine the significance of the differences between the means for the different groups. The results were regarded as significant when $P < 0.05$.

Results

Acetylcholine (3 nM–3 μM) caused contraction of the smooth muscle strips of the rainbow trout stomach in a concentration-dependent manner (ED_{50} 190 ± 40 nM, $n = 7$) but the contractile response declined in spite of the continued presence of acetylcholine in the organ bath. Tetrodotoxin (780 nM) did not affect the acetylcholine-induced contraction but atropine (500 nM) markedly reduced the contraction (Table 1). These data showed that acetylcholine acts on the muscarinic cholinoceptor present on the smooth muscle cell membrane.

Effects of noradrenaline

Noradrenaline caused a reproducible contractile response on 4 min exposure at 20 min intervals under the experimental conditions used. The contractile response to noradrenaline was non-sustained, with return to the base line within 3–4 min (Figure 1). A concentration-response curve for noradrenaline was obtained for the concentration range of 10 nM–10 μM (Figure 2). The ED_{50} value was 570 ± 120 nM ($n = 10$), and the maximum amplitude of the contraction was 73.1 ± 4.6% ($n = 10$) of that caused by 50 mM KCl. Noradrenaline did not cause any relaxation even after decline of its contraction, but papaverine (100 μM) relaxed all the preparations examined indicating that it has some spontaneous tone. The contractile action of noradrenaline was antagonized by phentolamine (5.4 μM), but not by a β-adrenoceptor blocking agent, carteolol (5 μM) (Figure 1). Isoprenaline, an agonist for β-adrenoceptors, relaxed the stomach strip and this relaxation was blocked by carteolol (5 μM) (Figure 1). This suggested that α-adrenoceptors were involved in the contractile response to noradrenaline while β-adrenoceptors could mediate relaxation, though not to noradrenaline.

Adrenoceptor subtype

Since phentolamine exhibits both α_1- and α_2-adrenoceptor blocking activity (Borowski *et al.*, 1977), it does not discriminate between α-adrenoceptor subtypes. Therefore, the contractile response to noradrenaline was investigated in the presence of yohimbine or prazosin. Yohimbine and prazosin did not cause any mechanical response of stomach strips and had no effects on the contraction induced by acetylcholine within the concentration-range examined.

Yohimbine, in the concentration-range of

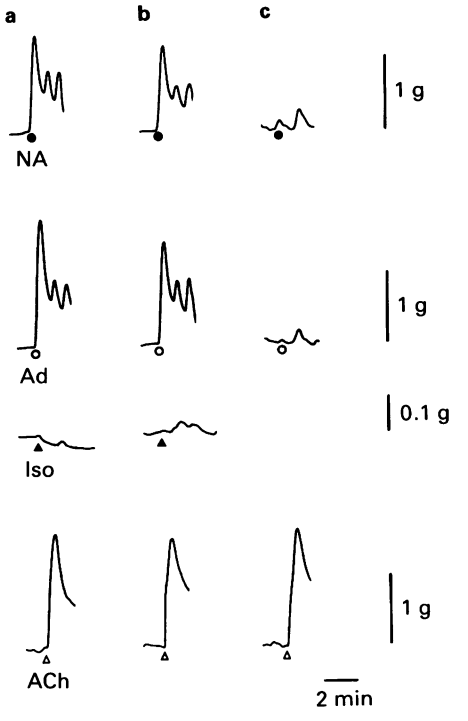

Figure 1 Typical mechanical responses induced by noradrenaline, adrenaline, isoprenaline and acetylcholine on smooth muscle strips isolated from rainbow trout stomach. The effects of noradrenaline (NA, ●, 1 μM), adrenaline (Ad, ○, 1 μM), isoprenaline (Iso, ▲, 10 μM) and acetylcholine (ACh, △, 300 nM) were examined in normal Krebs solution (a), and in the presence of carteolol (5 μM, b) or phentolamine (5.4 μM, c).

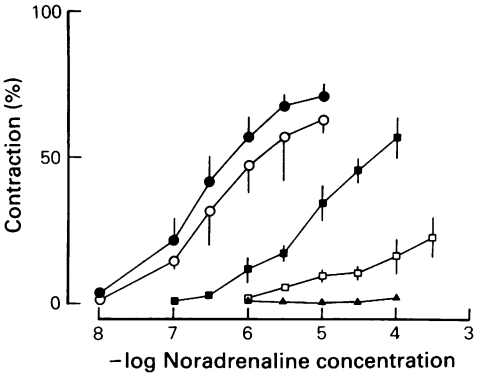

Figure 2 Effects of prazosin and yohimbine on the contraction induced by noradrenaline on smooth muscle strips isolated from rainbow trout stomach. The relationship between the concentration of noradrenaline and the contractile response in normal Krebs solution is shown (●). After 30 min pretreatment with prazosin (○, 1 μM) or yohimbine (■, 10 nM; □, 100 nM; ▲, 1 μM), dose-response curves for noradrenaline were obtained. Abscissa scale: concentration of noradrenaline (−log M). Ordinate scale: amplitude of the contraction expressed as a percentage of that induced by 50 mM KCl. Points represent the means of over six experiments with s.e.mean shown by vertical lines.

10 nM–1 μM, decreased the contraction induced by noradrenaline, and shifted the concentration-response curve to the right and downward. Prazosin caused a small shift to the right of the mean concentration-response curve for noradrenaline, but the effect was not statistically significant (Figure 2).

Effects of adrenaline, phenylephrine and methoxamine

Adrenaline, added to the organ bath at 20 min intervals, also caused a non-sustained concentration-dependent contraction which was reduced by phentolamine (5.4 μM) (Figures 1 and 3). The lowest concentration of adrenaline that caused contraction was 10 nM and the maximum contraction was obtained at 3 μM. The ED_{50} value was 280 ± 80 nM ($n = 8$), and the maximum amplitude of the contraction was 71.4 ± 5.1% ($n = 8$) of that caused by 50 mM KCl. The ED_{50} value of adrenaline was slightly lower than that of noradrenaline, but the maximum response to adrenaline was the same as that to noradrenaline. The contraction induced by adrenaline was concentration-dependently antagonized by yohimbine (10 nM–1 μM) (Figure 3).

Phenylephrine was less effective in contracting the stomach strips. A concentration-response curve was obtained for the range of 1 μM to 1 mM, and the amplitude of the maximum response was 18 ± 3.0% ($n = 7$) (Figure 4a). Yohimbine (1 μM) decreased the contraction induced by phenylephrine.

Methoxamine did not cause a contractile response, but, on the contrary, a high concentration of methoxamine (30 μM–100 μM) relaxed the preparations slightly (Figure 4b).

Effects of clonidine

Clonidine is known as a potent agonist of α_2-adrenoceptors (Drew, 1978; Tanaka & Starke, 1979; Starke, 1981). Clonidine (100 nM–300 μM) added to the organ bath solution did not cause any mechanical change (contraction or relaxation of a strip), while noradrenaline or acetylcholine usually caused marked contraction of the same preparation (Figure 4c).

In some preparations, we tried to obtain the concen-

tration-response curve for noradrenaline after washing out clonidine from the organ bath. The contractile response to noradrenaline obtained at 30 min after removal of clonidine was smaller than the control response obtained before clonidine application. Therefore, the interaction of clonidine and contraction induced by noradrenaline was investigated. Pretreatment for 10 min with clonidine (100 nM–1 μM) reduced the contraction induced by noradrenaline (Figure 5a). After washout, about 1 h was required for the responsiveness of smooth muscle to noradrenaline to recover to the control level. Figure 5b shows the dose-response curve for noradrenaline in the presence of clonidine (100 nM and 1 μM). Clonidine inhibited the contraction induced by noradrenaline in a non-competitive manner. Pretreatment with a low dose of noradrenaline (10 nM and 30 nM) did not affect the contraction induced by noradrenaline (1 μM), therefore it is unlikely that α-agonist pretreatment decreased the responsiveness of the smooth muscle to noradrenaline.

Effects of pretreatment with methoxamine (for 10 min) on the contraction induced by noradrenaline were also examined. Methoxamine (100 nM–10 μM) did not inhibit the contraction induced by noradrenaline. This suggested that the inhibitory effect was not due to an α_1-adrenoceptor agonist activity of clonidine.

To determine the specificity of the action of clonidine, its effects on the contraction induced by noradrenaline, adrenaline, acetylcholine and 5-hydroxytryptamine (5-HT) were examined in the same preparation. 5-HT (3 nM–3 μM) caused a concentration-dependent contraction of smooth muscle. Bath application of clonidine (1 μM for 10 min) markedly inhibited the contraction induced by noradrenaline or adrenaline but not that induced by acetylcholine or 5-HT (Figure 5a).

Effects of tetrodotoxin and atropine on the contraction induced by noradrenaline or adrenaline

Tetrodotoxin (780 nM), which abolished the response induced by transmural stimulation of a stomach strip (see below, Figure 6), partially reduced the contractile response induced by noradrenaline or adrenaline (Table 1). On the other hand, contractile responses to acetylcholine and 5-HT were not affected by tetrodotoxin (Table 1). The tetrodotoxin-resistant contraction induced by noradrenaline or adrenaline was decreased by pretreatment with yohimbine (1 μM). To clarify the nature of the tetrodotoxin-sensitive components, the effect of atropine was studied. Atropine (500 nM) caused a parallel shift to the right of the concentration-response curve for acetylcholine without affecting the maximum contraction. Compared with the control, the ED_{50} value for acetylcholine increased 2600 fold (ED_{50}: 500 ± 140 μM, $n = 6$). However, atropine at the same concentration did not decrease the contraction induced by noradrenaline or adrenaline significantly (Table 1).

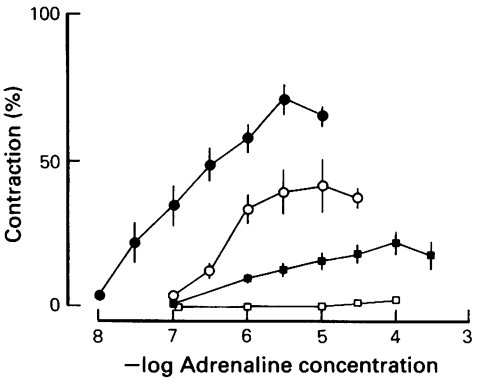

Figure 3 Effect of yohimbine on the contraction induced by adrenaline on smooth muscle strips isolated from rainbow trout stomach. The relationship between the concentration of adrenaline and the contractile response in normal Krebs solution is shown (●). After 30 min pretreatment with various concentrations of yohimbine (○, 10 nM; ■, 100 nM; □, 1 μM), dose-response curves for adrenaline were obtained. Abscissa scale: concentration of adrenaline (−log M). Ordinate scale: amplitude of the contraction expressed as a percentage of that induced by 50 mM KCl. Points represent the means of over six experiments with s.e.mean shown by vertical lines.

Effects of transmural stimulation

The results of the preceding experiments suggested that a part of the contraction induced by noradrenaline or adrenaline was the result of activation of a non-cholinergic excitatory nerve. Therefore, to determine whether or not a non-cholinergic nerve element was present, preparations were electrically stimulated. Transmural stimulation caused frequency-dependent contractions (Figure 6). Stimulation at 0.5 Hz was effective and stimulation at 20 Hz caused a near maximal response (Figure 6). The contractions were completely abolished by tetrodotoxin (Figure 6). Hexamethonium did not affect the contractions even at the high concentration of 100 μM. Atropine (1 μM) significantly reduced the contractions induced by low-frequency stimulation (0.5 Hz–2 Hz) but did not affect the contractions induced by high-frequency stimulation (3 Hz–20 Hz) (Figure 6).

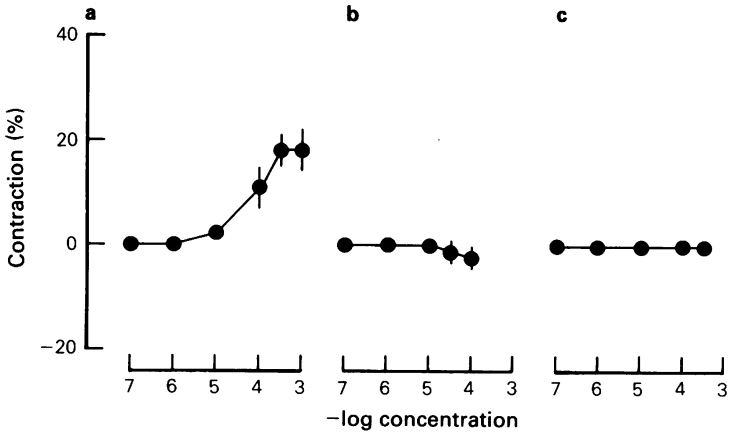

Figure 4 Relationship between the concentrations of phenylephrine, methoxamine and clonidine, and their responses on smooth muscle strips isolated from rainbow trout stomach. The graphs show the concentration-response curves for phenylephrine (a), methoxamine (b) and clonidine (c) in normal Krebs solution. Abscissa scale: concentrations of the three drugs (− log M). Ordinate scale: amplitude of the contraction expressed as a percentage of that induced by 50 mM KCl. Points represent the means of five experiments with s.e.mean shown by vertical lines.

Discussion

The results of the present study have confirmed the findings of Burnstock (1958), and Campbell & Gannon (1976) that noradrenaline and adrenaline cause non-sustained contraction of the trout stomach and that isoprenaline does not cause contraction. However, they did not determine the adrenoceptor subtype involved.

Since the contraction induced by noradrenaline or

Table 1 Effects of tetrodotoxin and atropine on the contraction of rainbow trout stomach strips induced by acetylcholine, 5-hydroxytryptamine, noradrenaline and adrenaline

		Relative contraction (%)		
		Control	*Tetrodotoxin* (780 nM)	*Atropine* (500 nM)
Acetylcholine	100 nM	39.5 ± 6.8 (7)*	41.7 ± 7.6 (5)	0 ± 0 ** (4)
	1 μM	95.8 ± 6.5 (7)	92.5 ± 11.7 (5)	0.5 ± 0.4** (5)
5-Hydroxytryptamine	100 nM	44.0 ± 8.2 (7)	49.4 ± 3.2 (5)	38.0 ± 3.4 (5)
	1 μM	77.8 ± 5.8 (7)	84.8 ± 2.6 (5)	80.3 ± 8.6 (4)
Noradrenaline	1 μM	59.4 ± 5.8 (11)	8.4 ± 3.3** (6)	58.4 ± 7.7 (5)
	3 μM	69.4 ± 4.2 (11)	24.8 ± 7.3** (6)	70.5 ± 8.3 (5)
	10 μM	76.4 ± 4.2 (11)	25.4 ± 4.1** (6)	70.4 ± 6.5 (5)
Adrenaline	1 μM	58.2 ± 4.7 (10)	23.3 ± 5.3** (6)	56.4 ± 10.6 (5)
	3 μM	71.7 ± 5.1 (10)	37.1 ± 6.1** (6)	62.5 ± 7.7 (6)
	10 μM	66.0 ± 3.3 (10)	41.1 ± 6.5** (6)	64.8 ± 6.7 (6)

Values are means ± s.e.
Relative contraction is expressed as a percentage of that induced by 50 mM KCl; 50 mM KCl-induced contraction was not affected by tetrodotoxin and atropine.
*Number of preparations examined.
**Significantly different from the control, $P<0.01$.

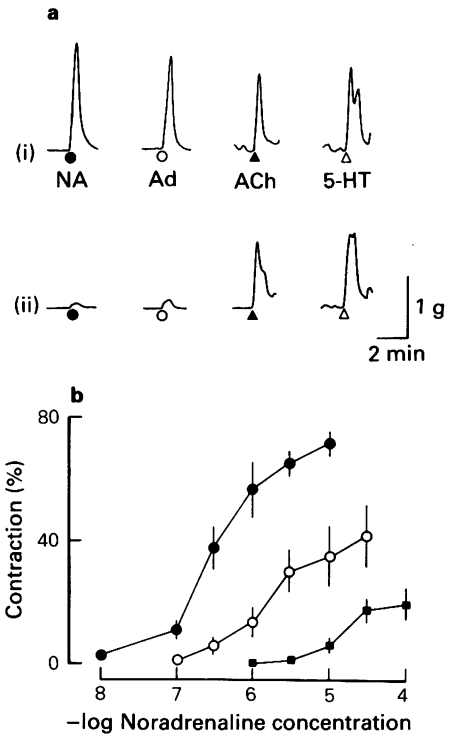

Figure 5 Inhibitory effect of clonidine on the contraction induced by noradrenaline on smooth muscle strips isolated from rainbow trout stomach (a). Contractile responses induced by noradrenaline (NA, ●, 2 μM), adrenaline (Ad, ○, 2 μM), acetylcholine (ACh, ▲, 1 μM) and 5-hydroxytryptamine (5-HT, △, 300 nM) in normal Krebs solution (i), and in the presence of clonidine (ii, 1 μM). (b) Relationship between the concentration of noradrenaline and the contraction in normal Krebs solution (●), and in the presence of clonidine (○, 100 nM; ■, 1 μM). Abscissa scale: concentration of noradrenaline (−log M). Ordinate scale: amplitude of the contraction expressed as a percentage of that induced by 50 mM KCl. Points represent the means of over five experiments with s.e.mean shown by vertical lines.

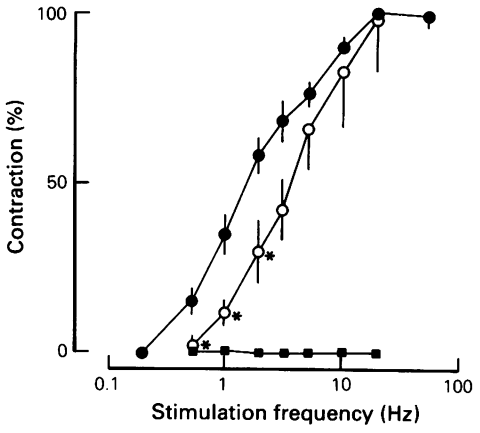

Figure 6 Effects of atropine and tetrodotoxin on the contraction induced by electrical transmural stimulation of smooth muscle strips isolated from rainbow trout stomach. The relationship between the stimulation frequency and the contraction induced by transmural stimulation in normal Krebs solution (●), and in the presence of atropine (○, 1 μM) and tetrodotoxin (■, 780 nM) is shown. Abscissa scale: stimulation frequency (Hz). Ordinate scale: amplitude of the contraction expressed as a percentage of the contraction induced by 20 Hz stimulation in normal Krebs solution. Points represent the means of five experiments with s.e.mean shown by vertical lines. *Significant difference from the control group, $P < 0.05$.

adrenaline was antagonized by phentolamine but not by carteolol, α-adrenoceptors are assumed to mediate the response. Furthermore, the α-adrenoceptors mediating the contraction seem to be of the α_2-type, because (1) prazosin, an antagonist with α_1-adrenoceptor blocking activity (Cambridge *et al.*, 1977; Doxey *et al.*, 1977), did not reduce the contraction induced by noradrenaline or adrenaline even at 1 μM, and (2) yohimbine, an antagonist with α_2-adrenoceptor blocking activity (Starke *et al.*, 1975; Weitzell *et al.*, 1979; Goldberg & Robertson, 1983) significantly reduced or abolished the contractions. This conclusion is further supported by the fact that phenylephrine and methoxamine, relatively selective α_1-adrenoceptor agonists (Drew, 1976; Van Meel *et al.*, 1981), were less effective in causing contraction.

An interesting aspect of the present experiments was that clonidine could not cause non-sustained contraction as noradrenaline or adrenaline did. Instead, clonidine reduced the contraction induced by noradrenaline or adrenaline without affecting the contraction induced by acetylcholine or 5-HT. As clonidine lacked the ability to cause contraction even at high concentrations and inhibited the contraction induced by α_2-adrenoceptor activation selectively, it was considered to act as an α_2-antagonist rather than an α_2-agonist in this preparation. The observation that clonidine acts as pure antagonist has also been made in cod coeliac artery (Johansson, 1979). Since clonidine is known as an α_2-adrenoceptor agonist in mammals (Drew, 1978; Tanaka & Starke, 1979; Starke, 1981),

the present results indicate that the α_2-adrenoceptor in the rainbow trout is somewhat different from that in mammals. The indications that α-adrenoceptors differ in fish and mammals are further supported by the fact that in cod spleen strips phenylephrine had a low intrinsic activity (Holmgren & Nilsson, 1974) and methoxamine produced very weak and irregular responses (Holmgren & Nilsson, 1976). However, further work, including radioisotope binding studies, is obviously needed to clarify the differences in the properties of fish and mammalian adrenoceptors and to determine if the inhibitory effect is specific to clonidine or shared with other imidazolines or structural analogues, e.g. xylazine and guanabenz.

Nilsson & Fänge (1969) reported that in the cod stomach the contractile effects of sympathomimetic drugs were mediated by cholinergic neurones, because the responses were reduced by atropine. In the present preparation, as catecholamine-induced contraction was partially inhibited by tetrodotoxin, the catecholamines were assumed to cause contraction through a direct action on smooth muscle and through an indirect action mediated by neuronal components. Atropine, 500 nM, which increased the ED_{50} value of acetylcholine 2600 fold, did not reduce the contraction induced by catecholamines and therefore it seems that the contraction due to catecholamines was mediated by a non-cholinergic nerve and not by a cholinergic nerve. To examine the nature of neuronal components present in the rainbow trout stomach, the preparation was stimulated electrically. Transmural stimulation caused a frequency-dependent contraction which was completely abolished by tetrodotoxin and unaffected by hexamethonium. Therefore the response induced by transmural stimulation was the result of excitation of postganglionic nerves (unless the ganglionic transmission is muscarinic). Atropine significantly inhibited the contraction induced by low frequency transmural stimulation but did not significantly inhibit the contraction induced by high frequency transmural stimulation. These data indicate that low frequency stimulation mainly activates an excitatory cholinergic nerve and, on the other hand, high frequency stimulation might activate a non-cholinergic excitatory nerve, although the possibility that high frequency stimulation causes a high local concentration of acetylcholine which overcomes competitive blockade cannot be excluded.

Isoprenaline caused relaxation of the smooth muscle which was blocked by carteolol. Therefore inhibitory β-adrenoceptors were present on the smooth muscle cells, as previously described for some teleost fishes (Nilsson & Fänge, 1967; 1969; Campbell & Gannon, 1976). The amplitude of the relaxation was very low because of the low tone of the preparations.

In conclusion, noradrenaline or adrenaline can cause contraction of smooth muscle strips of rainbow trout stomach through the activation of α_2-adrenoceptors. The contractile response to noradrenaline and adrenaline involves both a direct action on the smooth muscle and an indirect action through a non-cholinergic excitatory nerve.

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