Effect of adrenoceptor agonists on striated muscle strips of the canine oesophagus

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1 Acute psychological stress, which could be related to the release of a large amount of catecholamines, may cause oesophageal motility disorders. Therefore, the aim of our study was to elucidate the influence of adrenoceptor agonists on the striated muscle portion of the oesophagus by use of isolated strips from dogs.

2 Contractions were evoked in isolated striated muscle strips by electrical field stimulation (1 pulse min⁻¹, 1 ms/pulse, submaximal voltage). The effects induced by administration of adrenoceptor agonists alone or in the presence of antagonists were tested to determine the nature of the adrenoceptors on this muscle preparation.

3 The administration of both the natural adrenoceptor agonists, adrenaline and noradrenaline, and the synthetic β -adrenoceptor agonists, isoprenaline ($\beta_1 + \beta_2$), dobutamine (β_1) or ritodrine (β_2), enhanced the amplitude of the contractions induced by electrical stimulation in a concentration-dependent manner. The maximum responses were 82.6 (adrenaline), 66.2 (noradrenaline), 86.2 (isoprenaline), 34.6 (dobutamine) and 80.8% (ritodrine). The EC₂₀ values obtained were respectively 2 nM, 0.2 μ M, 0.91 nM, 3 μ M and 80 nM. The administration of the α_1 -adrenoceptor agonist, phenylephrine, also enhanced the contractile response in a concentration-dependent manner (EC₂₀ value = 0.3 μ M) and the maximum response was 64.6%, but the administration of the α_2 -adrenoceptor agonist, clonidine, did not influence the contractile response. These data suggest the involvement of β_2 - and possibly α_1 -adrenoceptors in the responses of these adrenoceptor agonists.

4 The selective β_2 -adrenoceptor antagonist ICI 118551 (3–100 nM) shifted the concentration-effect curves for noradrenaline, phenylephrine and ritodrine to the right in a concentration-dependent manner. ICI 118551 (3 nM) also shifted the concentration-effect curves for adrenaline and isoprenaline to the right, but increasing the concentration of ICI 118551 did not cause any further antagonist activity until a concentration of 100 nM, when a further rightward shift was obtained.

5 The selective α_1 -adrenoceptor antagonist, prazosin (30–300 nM), did not affect the increased contractile responses induced by adrenaline, noradrenaline, phenylephrine, isoprenaline or ritodrine.

6 In conclusion, it appears that β_2 -adrenoceptors are present in the striated muscle portion of the canine oesophagus, where they mediate an enhancement of contractile responses evoked by electrical stimulation. The α_1 -agonist, phenylephrine, appears to interact with β_2 -adrenoceptors on this preparation. β_3 -Adrenoceptors have already been demonstrated in smooth muscle from various parts of the gastrointestinal tract, and our study does not exclude the possibility that there is an additional population of β_3 -receptors in the canine striated muscle part of the oesophagus.

Keywords: Stress; striated muscle; oesophagus; β_2 -adrenoceptor; adrenoceptor agonists; contractile response

Introduction

Previous studies suggest that acute psychological stress causes oesophageal motility disorders. Cook and his colleagues (Cook *et al.*, 1987) have demonstrated that the resting pressure of the upper oesophageal sphincter (UES), which is composed entirely of striated muscle, was increased by a stressful listening task in human volunteers. Furthermore, the amplitude of the oesophageal contractions was increased in stressed normal subjects and in patients with noncardiac chest pain or the nutcracker oesophagus (Anderson *et al.*, 1989). Recent clinical studies indicate that high-amplitude peristaltic contractions or abnormal peristaltic sequences in the distal oesophagus could be related to symptoms of non-cardiac chest pain (Benjamin *et al.*, 1979; 1983; Peters *et al.*, 1988). In fact, it has been suggested that psychological characteristics, such as anxiety and depression, cause peristaltic abnormalities, an increase in wave amplitude, in wave duration or in frequency of abnormal peristaltic waves (Clouse & Lustman, 1983). However, how acute psychological stress

could induce such an abnormality of oesophageal function is incompletely understood.

Acute stress may release large amounts of catecholamines and adrenocorticotrophic hormone. Therefore, investigating the influence of adrenoceptor agonists on oesophageal function is important clinically. This has already been evaluated for the smooth muscle portion of the oesophagus. In the distal oesophageal body, α -adrenoceptor agonists enhance the contractile response (Christensen *et al.*, 1979; Tøttrup *et al.*, 1990), whereas β -adrenoceptor agonists inhibit the peristaltic contraction (Cohen, 1975; Dimarino & Cohen, 1982; Koch *et al.*, 1982; Lyrenas & Abrahamsson, 1986). Similar results have been obtained for the lower oesophageal sphincter (LES) (Dimarino & Cohen, 1975; Goyal & Rattan, 1978). Although many studies on the effects of adrenoceptor agonists on the smooth muscle portion of the oesophagus (including the LES) have been performed, only a few reports, describing the histology (Christensen & Percy, 1984; Marsh & Bieger, 1987) or *in vivo* experiments (Dodds *et al.*, 1979; Lyrenas & Abrahamsson, 1986), have been published on the adrenoceptor-mediated effects on the striated muscle portion. It has been suggested that neither α -adrenoceptor agonists

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nor β -adrenoceptor agonists induce an effect on the striated muscle portion of the opossum oesophagus *in vivo* (Dodds *et al.*, 1979). But the exact influence of adrenoceptor agonists on the striated muscle portion of the oesophagus is still unclear.

This *in vitro* study was therefore performed to investigate the effects of adrenoceptor agonists on oesophageal function by use of striated muscle strips isolated from dog oesophagus, and to attempt to elucidate the nature of the receptors involved.

Methods

Mongrel dogs of either sex were used for this experiment. After rapid removal of the upper oesophagus combined with the lower pharynx, this tissue was placed immediately in Krebs-Henseleit buffer at room temperature (composition in mM: KCl 4.69, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 2.51, NaHCO_3 25, KH_2PO_4 1.18, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.18, NaCl 118.06, glucose 5.55). The musculature of the oesophagus was freed from the adherent mucosa care being taken not to damage the underlying muscle layer; 8 longitudinal strips (length 4–5 cm, width 2 mm) were prepared from the zone directly below the pharyngo-oesophageal junction. Each strip was mounted between two platinum electrodes (8 cm long and 0.5 cm apart) and placed in a 100 ml organ bath filled with Krebs-Henseleit buffer, gassed with a mixture of 95% O_2 and 5% CO_2 and maintained at 37°C.

The strips were connected to an isometric force transducer under a preload of approximately 3 g, which had been previously evaluated as the mean optimal preload for this kind of preparation. The preparations were then allowed to stabilize for at least 40 min. After this stabilization procedure, electrical stimulation was applied over the entire length of the strip by means of 2 platinum electrodes (JSI power stimulator). The stimulation parameters were: 1 pulse min^{-1} , 1 ms/pulse. The voltage was increased by steps of 2 V, from 7 V up to 13 V. Contractions were measured isometrically (Statham UC2 force transducer) and recorded with a polygraph (BD9; Kipp & Zonen). When the maximum amplitude of the contraction was obtained, the voltage was reduced until a submaximal level (50% of the maximum amplitude) was reached. The strips were allowed to stabilize again for at least 15 min until reproducible twitch responses could be observed, after which drugs were administered to the organ bath.

In the first set of experiments, several adrenoceptor agonists were administered cumulatively to the organ bath: adrenaline (1 nM–3 μM), noradrenaline (30 nM–10 μM), phenylephrine (30 nM–30 μM), clonidine (300 nM–10 μM), isoprenaline (0.3 nM–1 μM), dobutamine (100 nM–30 μM) or ritodrine (3 nM–3 μM). In a control experiment, performed in parallel, similar preparations were treated with the appropriate type and amount (0.01–1.0 ml) of vehicle for each of the agonists.

In the next protocol, agonists were administered cumulatively in the presence of a single concentration of an antagonist (prazosin: 30 nM, 100 nM, 300 nM or 1 μM , or ICI 118551: 3 nM, 10 nM, 30 nM or 100 nM), or, in a control experiment, in the presence of solvent (distilled water). The effects which could be induced by the administration of the antagonists or solvent were followed for at least 15 min before administration of the agonists. The agonists adrenaline, noradrenaline, phenylephrine, isoprenaline and ritodrine were then administered in the same concentration ranges as in the first series.

Drugs

The following drugs were used: adrenaline, noradrenaline, phenylephrine and isoprenaline (Janssen Chimica, Belgium), clonidine (Boehringer, Germany), dobutamine (Shionogi,

Japan), ritodrine (Duphar, The Netherlands), prazosin (Pfizer, U.S.A.), ICI 118551 (1-[2,3-dihydro-4-methyl-1H-inden-7yl oxy]-3-[(1-methylethyl)amino]-2butanol hydrochloride, Imperial Chemical Industries, UK). Adrenaline was dissolved in 0.03% HCl. Noradrenaline and isoprenaline were dissolved in 0.9% NaCl. Ascorbic acid (2.5×10^{-7} M in the stock solution) was added to adrenaline, noradrenaline and the isoprenaline solution. The other drugs were dissolved in distilled water.

Figure 1 Effect of adrenoceptor agonists on contractions of the striated muscle strips of the canine oesophagus. (a) Concentration-response curves for adrenaline (○) and noradrenaline (●). (b) Concentration-response curves for phenylephrine (○) or clonidine (●). (c) Concentration-response curves for isoprenaline (○), ritodrine (●) and dobutamine (□). Mean $\pm$ s.e. mean (vertical lines) ($n = 5$) expressed as % increase of initial value.

Statistical analysis

All data are expressed for graphic presentation as mean percentage increase of initial values i.e. before the administration of the agonists (mean $\pm$ s.e.mean). Differences between mean values or EC_{20} values were tested with the ANOVA and Dunnett's *t* test. *P* values less than 0.05 were considered to indicate a significant effect.

For calculation of the EC_{20} value, the concentration of an agonist required to produce 20% of the maximal effect observed with isoprenaline was used.

EC_{20} values were calculated by linear regression analysis.

Results

Effect of adrenoceptor agonists on isolated striated muscle strips

Cumulative administration of adrenaline, noradrenaline, isoprenaline, dobutamine and phenylephrine enhanced the amplitude of the contractions elicited by electrical stimulation in a concentration-dependent manner whereas clonidine was without effect (see Figure 1). Typical responses to adrenaline, noradrenaline and phenylephrine are illustrated in Figure 2.

The rank order of agonist potency was: isoprenaline > adrenaline > ritodrine > noradrenaline > phenylephrine > dobutamine > clonidine. The EC_{20} values for the agonists had the same order of potency: isoprenaline [0.91 (0.65–1.3) nM], adrenaline [2 (1.4–2.7) nM], ritodrine [80 (44–130) nM], noradrenaline [0.17 (0.16–0.18) μ M], phenylephrine [0.3 (0.18–0.51) μ M], dobutamine [3 (2.0–4.7) μ M]. The maximal effects obtained were respectively: 86.2 $\pm$ 9%, 82.6 $\pm$ 6.3%, 80.8 $\pm$ 11.9%, 66.2 $\pm$ 8.1%, 64.6 $\pm$ 6.2%, 34.6 $\pm$ 5.1%.

Figure 2 Representative examples of the effects induced by adrenoceptor agonists on contractions of the isolated striated muscle strips of the canine oesophagus elicited by electrical stimulation (1 pulse min^{-1} , 1 ms/pulse, submaximal voltage).

Effect of adrenoceptor agonists in the presence of prazosin or ICI 118551 on isolated striated muscle strips

Addition of prazosin (up to 1 μ M) did not affect the contractile responses to electrical stimulation. Furthermore, in concentrations of 30–300 nM, prazosin had no effect on concentration-effect curves for adrenaline, noradrenaline, isoprenaline or ritodrine, and in concentrations of 100–1000 nM, it was similarly ineffective with reference to concentration-effect curves to phenylephrine (Figures 3a, 4a, 5a, 6a and 7a).

Addition of ICI 118551 (up to 100 nM) did not affect the contractile responses to electrical stimulation. However, ICI 118551 significantly shifted to the right the concentration-effect curves for adrenaline, noradrenaline, phenylephrine, isoprenaline or ritodrine (Figures 3b, 4b, 5b, 6b and 7b). This antagonism was associated with some apparent flattening of the agonist curves.

ICI 118551 inhibited the response to noradrenaline, phenylephrine and ritodrine in a concentration-dependent manner, whereas its effect on adrenaline and isoprenaline was not concentration-dependent. With adrenaline and isoprenaline, the lowest concentration of ICI 118551 (3 nM) caused a marked rightward shift of agonist concentration-effect curves, but increasing the concentration to 10 and 30 nM caused no further shift. Only when a concentration of ICI 118551 of 100 nM was achieved was a further rightward shift obtained.

Figure 3 Effect of adrenaline on contractions of the striated muscle strips of the canine oesophagus. (a) Concentration-response curves for adrenaline in the presence of solvent (O), 30 nM prazosin (●), 100 nM prazosin (□) and 300 nM prazosin (■). (b) Concentration-response curves for adrenaline in the presence of solvent (O), 3 nM ICI 118551 (●), 10 nM ICI 118551 (□), 30 nM ICI 118551 (■) and 100 nM ICI 118551 (Δ). Mean $\pm$ s.e.mean (vertical lines) (*n* = 5) expressed as % increase of initial value.

Figure 4 Effect of noradrenaline on contractions of the striated muscle strips of the canine oesophagus. (a) Concentration-response curves for noradrenaline in the presence of solvent (○), 30 nM prazosin (●), 100 nM prazosin (□) and 300 nM prazosin (■). (b) Concentration-response curves for noradrenaline in the presence of solvent (○), 3 nM ICI 118551 (●), 10 nM ICI 118551 (□), 30 nM ICI 118551 (■) and 100 nM ICI 118551 (Δ). Mean $\pm$ s.e.mean (vertical lines) ($n = 5$) expressed as % increase of initial value.

Figure 5 Effect of phenylephrine on contractions of the striated muscle strips of the canine oesophagus. (a) Concentration-response curves for phenylephrine in the presence of solvent (○), 100 nM prazosin (●), 300 nM prazosin (□) and 1 μM prazosin (■). (b) Concentration-response curves for phenylephrine in the presence of solvent (○), 3 nM ICI 118551 (●), 10 nM ICI 118551 (□), 30 nM ICI 118551 (■) and 100 nM ICI 118551 (Δ). Mean $\pm$ s.e.mean (vertical lines) ($n = 5$) expressed as % increase of initial value.

Figure 6 Effect of isoprenaline on contractions of the striated muscle strips of the canine oesophagus. (a) Concentration-response curves for isoprenaline in the presence of solvent (○), 30 nM prazosin (●), 100 nM prazosin (□) and 300 nM prazosin (■). (b) Concentration-response curves for isoprenaline in the presence of solvent (○), 3 nM ICI 118551 (●), 10 nM ICI 118551 (□), 30 nM ICI 118551 (■) and 100 nM ICI 118551 (Δ). Mean $\pm$ s.e.mean (vertical lines) ($n = 5$) expressed as % increase of initial value.

Figure 7 Effect of ritodrine on contractions of the striated muscle strips of the canine oesophagus. (a) Concentration-response curves for ritodrine in the presence of solvent (O), 30 nM prazosin (●), 100 nM prazosin (□) and 300 nM prazosin (■). (b) Concentration-response curves for ritodrine in the presence of solvent (O), 3 nM ICI 118551 (●), 10 nM ICI 118551 (□), 30 nM ICI 118551 (■) and 100 nM ICI 118551 (Δ). Mean $\pm$ s.e.mean (vertical lines) ($n=5$) expressed as % increase of initial value.

Discussion

The neural control mechanism of peristalsis in the striated muscle portion of the oesophagus differs from that in the smooth muscle portion (Roman & Gonella, 1987). In manometric studies, however, peristalsis induced in the different portions shows a similar coordinated profile. Two control mechanisms for peristalsis exist in the smooth muscle portion: one is the central control mechanism mediated via the vagal nerve and the other is the peripheral control mechanism which can be regulated by the myenteric plexus (Diamant & El-Sharkawy, 1977). The myenteric plexus is also present in the striated muscle portion, although less well developed (Diamant, 1989). Therefore, the action of this muscle portion might be modulated mainly by extrinsic nerves (Roman & Gonella, 1987).

The importance of the cholinergic effects in the striated muscle portion of the oesophagus has been demonstrated extensively (Dodds *et al.*, 1979; Crist *et al.*, 1984; Marsh & Bieger, 1987; Taylor, 1990).

Concerning the adrenoceptor, previous studies *in vivo* and *in vitro* have demonstrated that α -adrenoceptor agonists enhance the contractile response of the LES to electrical stimulation. β -Agonists reduce the contractile response. None of the adrenoceptor agonists influence the relaxation of the LES (Dimarino & Cohen, 1975; Goyal & Rattan, 1978). In

the striated muscle portion, however, it has been demonstrated that phenylephrine and isoprenaline do not induce any enhancement or inhibition in the peristaltic contraction of the opossum oesophagus. There is no evidence for ganglionic synapses in the neural pathway (Dodds *et al.*, 1979). Our study was designed to elucidate the action of adrenoceptor agonists on the striated muscle portion by the use of isolated oesophageal strips of dogs as a model.

Adrenaline and noradrenaline, which have agonist activity at both α - and β -adrenoceptors, enhanced the contractile responses, noradrenaline being less potent than adrenaline. This suggests that the effect is induced via β -adrenoceptors, since adrenaline has been shown to be a more potent β -agonist than noradrenaline and a less potent α -agonist. A range of agonists specific for α - and β -adrenoceptors were also tested in an attempt to identify the subtype of the adrenoceptor involved. The results, obtained with the selective α_1 -agonist phenylephrine or the selective α_2 -agonist clonidine, suggest that α_1 -adrenoceptors are involved. However, in other experiments we conducted, a range of β -adrenoceptor agonists, isoprenaline ($\beta_1 + \beta_2$ -agonist), dobutamine (selective β_1 -agonist) and ritodrine (selective β_2 -agonist) were tested; ritodrine increased the contractile response, suggesting the presence of β_2 -adrenoceptors on the striated muscle portion. On the other hand, dobutamine only moderately increased the contractile response and only at high concentrations. It is known that high concentrations of dobutamine can activate both β_1 - and β_2 -adrenoceptors (Hoffman & Lefkowitz, 1990). The results from this series of experiments are consistent with the presence of β_2 -adrenoceptors, and there is no convincing evidence for the involvement of β_1 -adrenoceptors.

In another series of experiments, agonists were administered cumulatively in the presence of the selective α_1 -antagonist prazosin (Berthelsen & Pettinger, 1977; Doxey *et al.*, 1977) or the selective β_2 -antagonist ICI 118551 (Bilski *et al.*, 1980; Wilffert *et al.*, 1982a). Whereas prazosin did not block any of the agonists, ICI 118551 inhibited the increases in contractile responses induced by all of the agonists tested.

The mechanism by which ICI 118551 inhibits the effect induced by phenylephrine is unclear. In vascular smooth muscle, ICI 118551 did not influence the electrical stimulation-induced increase in diastolic pressure which was strongly attenuated by the α_1 -antagonist prazosin (Wilffert *et al.*, 1982b), suggesting that ICI 118551 is devoid of α_1 blocking properties. We, therefore, may suppose that phenylephrine acts via a β_2 -adrenoceptor in the striated muscle portion of the oesophagus. Stimulation of β -adrenoceptors by phenylephrine has been observed, but only at a much higher concentration (Hoffman & Lefkowitz, 1990). Therefore, the interaction of ICI 118551 with the responses to phenylephrine is still difficult to explain.

On the other hand, addition of ICI 118551 did not induce a concentration-related antagonism. The effects induced after addition of adrenaline and isoprenaline, and also to a degree after addition of phenylephrine, could be reduced in the presence of 3 nM ICI 118551. At 10 and 30 nM ICI 118551, no further effect was observed. Only after addition of 100 nM could a further antagonism be seen. These results could be explained by the presence of a mixed receptor population, one receptor type being blocked by the lower concentrations of ICI 118551, the other only sensitive to blockade by the highest concentration tested (100 nM). Atypical or β_3 -adrenoceptors have already been demonstrated in the smooth muscle part of the guinea-pig ileum (Taneja & Clarke, 1992), in the rat ileum (Van der Vliet *et al.*, 1990) and in the rat colon (McLaughlin & MacDonald, 1990) but they mediate inhibitory activity on smooth muscle. It is possible that they also exist in canine oesophagus and that they mediate an excitatory effect. More experiments, however, are needed to confirm the importance of the β_3 -receptor in this model.

In conclusion, in our study adrenaline, noradrenaline, phenylephrine, isoprenaline and ritodrine enhanced the con-

tractile response of isolated striated muscle strips, an action which was suppressed by low concentrations of the β_2 -adrenoceptor antagonist ICI 118551. These results suggest that facilitatory β_2 -adrenoceptors are present in the striated muscle portion of the oesophagus, and that in this region the α_1 -agonist phenylephrine acts via β_2 -adrenoceptors. We hypo-

thesize that acute psychological stress may cause oesophageal motility disorders via adrenoceptors, predominantly of the β_2 -subtype in the striated muscle portion of the oesophagus.

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