Original article

L-Erythro-methoxamine is more potent than phenylephrine in effecting contraction of internal anal sphincter *in vitro*

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Background: Topical phenylephrine has been shown to increase resting anal canal pressure in normal and incontinent individuals. However, high concentrations of gel (10–40 per cent) are required that may cause local side-effects. The aim of this study was to determine whether methoxamine, another α-1-adrenoceptor agonist, might be a more potent alternative to phenylephrine.

Methods: Porcine internal anal sphincter (IAS) tissue was cut into strips, suspended in a superfusion organ bath and allowed to equilibrate. Strips were subjected to each drug under test for 20 s, sufficient to obtain stable tone. Phenylephrine, methoxamine (1 : 1 : 1 : 1 ratio of its four isomers) and each of the individual isomers of methoxamine were evaluated in turn.

Results: *In vitro*, methoxamine racemate and phenylephrine were similarly potent in causing contraction of IAS strips (mean(s.e.m.) dose giving half maximal effect (EC_{50}) at 74·7(16·5) *versus* 58·3(13·4) μM respectively; $P = 0{\cdot}443$). However, one of the methoxamine isomers, L-erythro-methoxamine (EC_{50} 17·6(3·7) μM), was significantly more potent than the other three isomers, methoxamine racemate and phenylephrine ($P = 0{\cdot}002$).

Conclusion: L-Erythro-methoxamine is four times more potent than phenylephrine and is a possible treatment for incontinence. Trials are under way to examine the efficacy of L-erythro-methoxamine *in vivo*.

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Introduction

The most common cause of faecal incontinence is damage to the anal sphincter complex either through pudendal neuropathy or direct trauma during childbirth[1]. Faecal incontinence may also be seen in the absence of structural injury; in such circumstances, isolated degeneration of the internal anal sphincter (IAS) is the most common cause[2].

Conservative measures for mild symptoms of incontinence include the use of pads[3] or plugs[4], antidiarrhoeal medication[5] and dietary modification. Damage to the external anal sphincter may be amenable to overlapping surgical repair[6] but results of IAS repair have been disappointing[7]. More extensive surgical procedures do exist for profound damage, including the artificial bowel sphincter[2], sacral nerve stimulation[8] and graciloplasty[9], but these are major interventions and may be either unsuitable or poorly tolerated by many patients.

Recent advances in understanding of the pharmacology of the anorectum have resulted in the development of potential drug therapies for faecal incontinence. The IAS receives excitatory, sympathetic innervation, mediating contraction through an action on α-adrenoceptors[10]. This study was an analysis of the potency of methoxamine racemate and its four stereoisomers compared with phenylephrine on porcine IAS muscle strips *in vitro*.

Materials and methods

Tissue from female Large White pigs was collected from a local abattoir. Anal canal tissue was trimmed and transferred to cold Krebs' solution at 4°C. Krebs' solution comprised sodium chloride 120 mmol/l, potassium chloride 5·9 mmol/l, sodium bicarbonate 15·4 mmol/l, monosodium phosphate 1·2 mmol/l, calcium chloride

2·5 mmol/l, magnesium chloride 1·2 mmol/l and glucose 11·5 mmol/l. This was bubbled with 97 per cent oxygen/3 per cent carbon dioxide.

Specimens were pinned to a silicon elastomer in the base of a petri dish containing cold Krebs' solution at 4°C. With the aid of a dissecting microscope, the mucosa and submucosa were removed. Strips of smooth muscle were cut from the IAS. The approximate dimension of the muscle strips was 7 × 1 × 1 mm and the weight of each strip was typically 3–7 mg. Strips were cut in an orientation such that they contained parallel smooth muscle bundles. Ligatures of 5/0 silk were tied to each end of the strip for mounting in a superfusion organ bath. One end was fixed and the other attached to an isometric tension transducer.

Smooth muscle strips were mounted in 0·2-ml perspex organ baths between two recessed platinum electrodes through which electrical field stimulation could be delivered. The strips were perfused constantly with Krebs' solution at 37°C at a rate of 1 ml/min. The solution was bubbled with 97 per cent oxygen/3 per cent carbon dioxide. The apparatus comprised six organ baths in parallel, allowing the study of six strips simultaneously[11].

Force generated by the smooth muscle strips was measured using Pioden Dynamometer UFI transducers™ (Pioden Controls, Canterbury, UK), amplified (Harvard Transducer/Amplifier™; Harvard Apparatus, Edenbridge, UK) and recorded using MacLab Data Acquisition™ (AD Instruments, Sydney, New South Wales, Australia) and Chart v3·6™ software (Apple, Cupertino, California, USA).

The equipment was calibrated before each experiment using a 1-g weight attached to the transducer. After strips had been mounted, they were stretched with an initial force of 1 g, and allowed to equilibrate for at least 1 h before starting the experiments. At the conclusion of experiments, strips were exposed to calcium-free Krebs' solution, in which the calcium chloride was replaced iso-osmotically with magnesium chloride and to which the calcium chelator EGTA 0·5 mmol/l was added. Passive tension, measured in calcium-free Krebs' solution, was subtracted from observed tension to give actual tension.

Drugs used were phenylephrine (Sigma Chemical, Poole, UK) and methoxamine racemate (1 : 1 : 1 : 1 ratio of its four stereoisomers; Norgine Research, Northwood, UK) and its four stereoisomers (Advanced Separation Technologies, Congleton, UK). The four stereoisomers were obtained by chromatographic separation of the 1 : 1 : 1 : 1 racemate, and were initially denoted peaks 1, 2, 3 and 4. All drugs were dissolved in Krebs' solution. A small air bubble was introduced as each drug was administered to ensure that there was minimal mixing and/or dilution of the drug by normal Krebs' solution. Increasing concentrations of drugs were added for 20-s intervals, with intervening washout periods of at least 10 min, until the tone had returned to baseline.

Drug-induced increases in tone were calculated by taking the peak tone after drug application and subtracting this from basal tone. This value was then expressed as a percentage of maximal increase in tone seen across the dose range used (10 mmol/l to 0·01 μmol/l). All analysis was performed using Chart v3·6 software™.

The dose causing 50 per cent of maximal contraction (EC_{50}) was calculated by plotting concentration–response curves for each muscle strip. EC_{50} values were calculated by linear regression and values for different drugs were compared using a two-tailed *t* test, assuming unequal variance. $P < 0{\cdot}050$ was considered significant.

Results

Methoxamine racemate and phenylephrine

Both methoxamine racemate and phenylephrine caused a dose-dependent contraction of smooth muscle strips from the IAS. This could be blocked by pretreatment of muscle strips with phentolamine, an α-adrenoceptor antagonist, indicating that both methoxamine and phenylephrine were acting via α-adrenoceptors. EC_{50} values for methoxamine racemate (58·3(13·4) μM) and phenylephrine (74·7(16·5) μM) did not differ significantly ($P = 0{\cdot}443$). Concentration–response curves are shown in *Fig. 1*. Each line is based on the mean of 24 muscle strips derived from four pigs, denoted $n = 24(4)$.

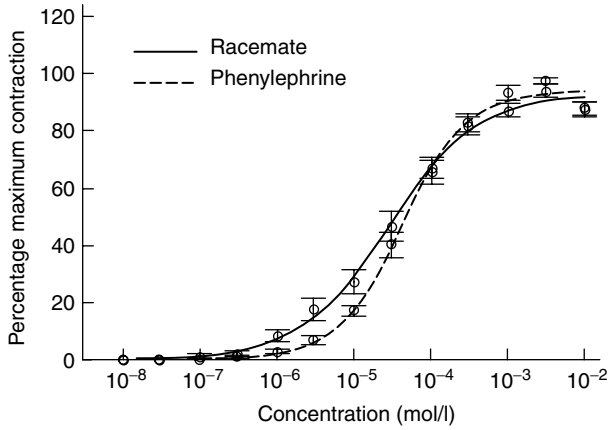

Fig. 1 Concentration–response curves for phenylephrine and methoxamine racemate. Values are mean(s.e.m.) ($n = 24(4)$). These curves did not differ significantly ($P = 0{\cdot}443$, two-tailed *t* test)

Methoxamine stereoisomers

All isomers of methoxamine caused dose-dependent contraction of IAS smooth muscle strips. EC_{50} values are shown in *Table 1*. Peak 2 was significantly more potent than peaks 1, 3 and 4 ($P < 0{\cdot}001$) (*Fig. 2*). Peak 2 was also significantly more potent than methoxamine racemate ($P = 0{\cdot}007$) and phenylephrine ($P = 0{\cdot}002$) (*Fig. 3*).

Peak 2 has been identified as L-erythro-methoxamine (data courtesy of Norgine Research Limited, Northwood, UK). A novel synthetic pathway for this agent has been developed, providing a second more abundant source of the isomer, referred to below as synthetic L-erythro-methoxamine.

Table 1 Dose of phenylephrine, methoxamine racemate and its isomers causing half maximal contraction of porcine internal anal sphincter

Drug	Racemate or isomer	EC_{50} (μM)
Phenylephrine	Racemate	74·7(16·5)
Methoxamine	Racemate	58·3(13·4)
	Peak 1	316·5(40·6)
	Peak 2	17·6(3·7)
	Peak 3	165·4(12·1)
	Peak 4	482·7(80·6)

Values are mean(s.e.m.) ($n = 24(4)$). Peak 2 corresponds to L-erythro-methoxamine. EC_{50}, dose causing half maximal contraction.

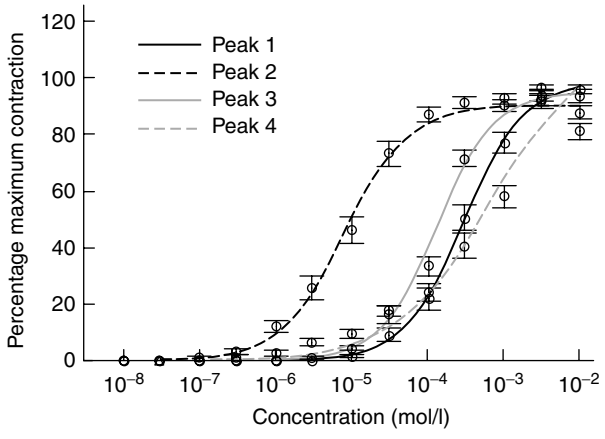

Fig. 2 Concentration–response curves for four stereoisomers of methoxamine. Values are mean(s.e.m.) ($n = 24(4)$). Peak 2 corresponds to L-erythro-methoxamine and was significantly more potent than peaks 1, 3 and 4 ($P < 0{\cdot}001$, two-tailed *t* test)

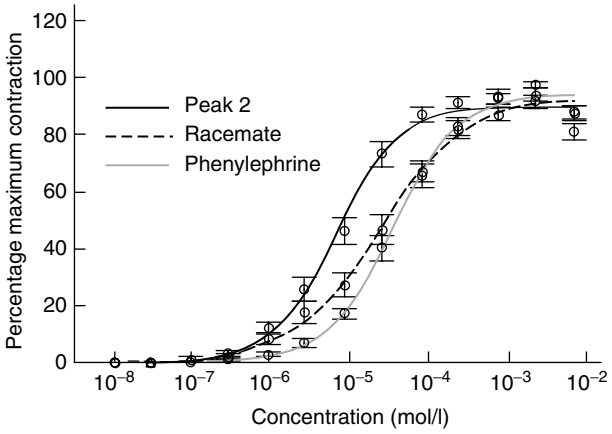

Fig. 3 Concentration–response curves for L-erythro-methoxamine (peak 2), methoxamine racemate and phenylephrine. Values are mean(s.e.m.) ($n = 24(4)$). Peak 2 (L-erythro-methoxamine) was significantly more potent than methoxamine racemate and phenylephrine ($P = 0{\cdot}007$ and $P = 0{\cdot}002$, respectively, two-tailed *t* test)

Synthetic L-erythro-methoxamine

Synthetic L-erythro-methoxamine also caused dose-dependent contraction of IAS strips (EC_{50} 10·5(1·97) μM), and was significantly more potent than peaks 1, 3 and 4 ($P < 0{\cdot}001$). The EC_{50} did not differ significantly from that of separated L-erythro-methoxamine ($P = 0{\cdot}101$) (*Fig. 4*). Synthetic L-erythro-methoxamine was significantly more potent than methoxamine racemate ($P = 0{\cdot}002$) and phenylephrine ($P = 0{\cdot}001$).

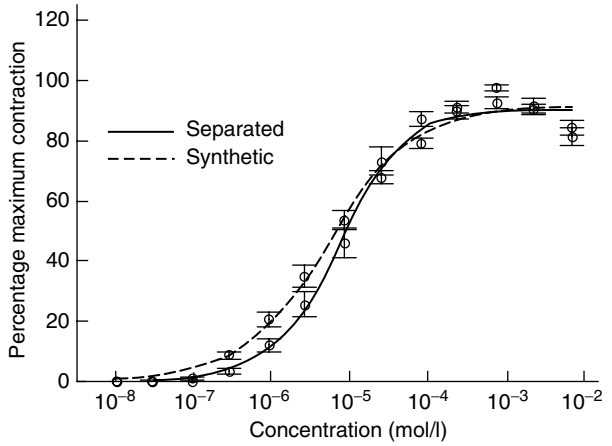

Fig. 4 Concentration–response curves for L-erythro-methoxamine (synthetic) and L-erythro-methoxamine (separated). Values are mean(s.e.m.) ($n = 24(4)$). L-Erythro-methoxamine corresponds to peak 2. These curves did not differ significantly ($P = 0{\cdot}101$, two-tailed *t* test)

Discussion

The use of topical agents for the treatment of faecal incontinence is a new approach to an old problem. To date, all clinical research on such topical therapies has been focused on phenylephrine, another α-1-adrenergic agonist. The present results suggest that L-erythro-methoxamine is a potent α-1-adrenergic agonist. Although these experiments were performed on porcine IAS tissue, previous work from this group has shown that porcine IAS is both qualitatively and quantitatively similar to human IAS tissue[12].

Topical phenylephrine has been shown to produce a dose-dependent rise in resting anal canal pressure in normal human subjects. At a concentration of 10 per cent, the gel produced a 33 per cent increase in pressure that was sustained for a median of 7 h[13]. However, in patients with an ultrasonographically normal anal sphincter, but low resting anal canal pressure and symptoms of incontinence, no significant rise in pressure was seen with 10 or 20 per cent gel, although 30 and 40 per cent gel produced a significant increase[14]. These data suggest that the IAS from patients with incontinence is less sensitive to adrenergic agonists *in vivo*. There is evidence to support this from *in vitro* studies[15]. Perhaps unsurprisingly, therefore, in a randomized controlled trial of 36 patients with incontinence and an ultrasonographically normal sphincter, no significant overall improvement was seen in incontinence scores, resting anal canal pressures or anodermal blood flow[16].

Future research should be directed at identifying which subgroup of incontinent patients might benefit from topical phenylephrine. For example, 10 per cent phenylephrine gel was found to produce a significantly greater subjective improvement in continence compared with placebo, in a small randomized clinical trial of patients with faecal leakage after ileoanal pouch construction[17].

The appropriate dose of topical phenylephrine to use in the treatment of incontinence has not yet been established. However, high concentrations, of the order of 30–40 per cent, may be required, at which a sensation of perianal burning has been reported[14]. Another theoretical concern is that high doses of topical α-adrenergic agonists might affect blood pressure or pulse rate. Although this has not proved problematic in clinical trials to date, cardiovascular side-effects are seen when phenylephrine is applied topically in ophthalmology[18]. Interestingly, local irritation is also seen when the drug is used for this indication[19].

Any effects of topical α-adrenergic agonists on cardiovascular variables are likely to represent little more than systemic absorption and α-adrenergic cardiovascular stimulation. By contrast, the local adverse effects seen with phenylephrine may not be mediated through α-adrenergic activation. As lower concentrations of L-erythro-methoxamine than phenylephrine are necessary to achieve similar effects on maximum anal resting pressure, it is hoped that these local adverse effects will prove less marked with L-erythro-methoxamine. *In vivo* studies are under way to establish whether L-erythro-methoxamine may be used safely as an effective topical agent without significant local and systemic adverse effects.

Acknowledgements

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