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Characterization of rat gastric myogenic contractions and modulation by oxytocin and arginine-vasopressin

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ABSTRACT

Background: Interstitial cells of Cajal generate slow wave gastric electrical activity, initiating spontaneous muscle contractions. This becomes dysrhythmic during nausea when [Arg^8]-vasopressin (AVP) is also released. In human stomach AVP increased spontaneous contraction activity and muscle tone, not neuronally-mediated contractions. Rodents cannot vomit, releasing the related hormone, oxytocin (OT) instead. We hypothesised that rat stomach would behave differently.

Experimental approach: Spontaneous and electrically-evoked (EFS) contractions were measured in rat forestomach and antrum circular muscle. Custom software defined spontaneous contractions by analysing eight motility parameters.

Results: The forestomach was quiescent. Irregular antrum contractions became regular adjacent to the pylorus (1.7 ± 0.4 mN; 1.2 ± 0.1 contractions/min, n = 12). These were unaffected by tetrodotoxin (10^{-6} M), atropine (10^{-6} M) and L-NAME (3×10^{-4} M). In both regions, AVP (pEC_{50}~9.0) and OT (~0.5 $\log_{10}$-unit less potent) caused contraction (greater in antrum), competitively antagonized by, respectively, SR49059 (pK_B~9.5) and L371257 (pK_B~9.0), reduced by tetrodotoxin but unaffected by atropine. In the antrum, AVP and OT (~2 $\log_{10}$-units less potent/efficacious) regularized and increased spontaneous contraction amplitude, frequency, rates of contraction/decay. In both regions, EFS-evoked contractions, abolished by atropine/tetrodotoxin, were reduced by AVP and OT, with AVP more potent and efficacious, particularly in forestomach.

Conclusion: Irregular spontaneous contractions of gastric antrum suggest variable ICC-muscle coupling. AVP and less potently, OT, enhanced frequency and force of contractions via V_{1A} and OT receptors. Compared with human, differences in contraction regularity, potency and ability of AVP/OT to affect neuronal function suggests caution when using rat stomach to model ICC functions and nauseagenic stimuli.

1. Introduction

An ability to empty stomach contents by vomiting exists throughout the Mammalia class, except among the Rodentia, exemplified by rats or mice, and the Lagomorpha, exemplified by the rabbit; as these orders evolved this function was lost, perhaps in part, to aid water conservation and perhaps related to different eating behaviours (Sanger et al., 2011). In response to stimuli which induce vomiting in humans, rats and mice exhibit behaviours such as taste aversion, pica consumption and conditioned gaping, sometimes defined as 'nausea-like' (Horn et al., 2013; Andrews and Sanger, 2014). However, since nausea is an experience that is self-expressed verbally by humans, it is impossible to know with certainty if these animal behaviours are indicative of the human experience, and if so, the extent to which they reflect the physiological changes that accompany nausea in humans.

In humans, nausea and vomiting, and the release of [Arg^8]-vasopressin (AVP) from the pituitary are associated with dysrhythmic gastric electrical activity; further, exogenously-administered AVP can itself induce nausea and gastric electrical dysrhythmia (Koch, 1997). Gastric electrical activity, usually assessed externally by electrogastrogram (EGG), largely reflects spontaneous depolarization of the interstitial cells of Cajal (ICC) in the stomach wall, creating slow waves of depolarization which pass circumferentially and down the stomach towards the pylorus (Sanders et al., 2006; O'Grady et al., 2010; Angeli et al., 2013). During nausea this pattern of activity becomes disrupted, either by disease and/or by endogenous substances influencing ICC functions, leading to hypomotility and retrograde movements within the stomach (O'Grady et al., 2021).

Slow wave electrical activity can initiate spontaneous contractions of

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Abbreviations

AP	Area Postrema
AVP	[Arg^8]-vasopressin
DMSO	Dimethyl sulfoxide
EGG	Electrogastrography
EFS	Electrical Field Stimulation
E_{max}	maximum response achieved from an applied agent
GI	Gastrointestinal
ICC	Interstitial Cell of Cajal
L-NAME	Nω-nitro-L-arginine methyl ester hydrochloride
OT	Oxytocin
NTS	*Nucleus Tractus Solitarius*
pA_2	negative $\log_{10}$ of the molar concentration of antagonist which reduces the effect of double concentration of an agonist to that of single concentration
pEC_{50}	negative $\log_{10}$ of the concentration of the agonist achieving half the maximal response
pK_B	negative $\log_{10}$ of the equilibrium dissociation constant of the antagonist-receptor complex
TTX	Tetrodotoxin

human isolated stomach muscle (Rhee et al., 2011). In the gastric antrum these contractions are not dependent on neuronal activity (and known as 'myogenic contractions'), occur at a frequency of approximately 3 contractions/minute, comparable to that measured *in vivo* (Makwana et al., 2022; Straface et al., 2022). The contractions are modulated by compounds influencing ICC functions (Straface et al., 2022) and are greatly increased (i.e., increased amplitude, frequency and rate of contraction) by low concentrations of AVP, in a manner consistent with stimulation of ICC activity and which in turn, synergized with similar effects of additional application of adrenaline (Makwana et al., 2022). Compared with humans, rodent gastric functions exhibit significant structural and physiological differences related to their different eating behaviours and loss of ability to vomit (e.g., in vagal-brainstem architecture, gastro-esophageal morphology, the pharmacology of 5-HT_3 and NK_1 receptors with key roles in mechanisms of vomiting, and in the release of oxytocin by rodents in response to 'emetic' stimuli, instead of AVP; see Sanger et al., 2011; Horn et al., 2013). Accordingly, we were interested to see how the spontaneous myogenic contractions of rat stomach compare with those in human stomach and whether these are similarly modified by AVP and oxytocin. Our hypothesis was that in a non-vomiting species, the gastric myogenic contractions, and/or their modulation by nauseagenic stimuli such as AVP (or oxytocin) would not be the same as in human.

2. Materials and methods

2.1. Animals

Animal care complied with the Animals Scientific Procedures Act (ASPA) 1986, the European Directive 20/63 provisions as transposed into the UK and parts of the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines (Percie du Sert et al., 2020) applicable to use of animal tissue harvested for *in vitro* research. Male and female virgin Sprague-Dawley rats (8–10 weeks old, 150–280g, Charles River Laboratories, UK) were segregated and housed in a room with controlled temperature (22 ± 1 °C), humidity (55 ± 10%) and 12h light-dark cycle for 6 days before use. Food (LabDiet® EURodent diet 14%, IPS Product Supplies, UK) and water was provided *ad libitum*. Females were in oestrus (determined by light microscopy of vaginal smears). Rats were killed by CO_2 asphyxiation followed by cervical dislocation and spinal cord transection to ensure death before stomach removal. The resected stomach was placed in Krebs-Henseleit solution (x10^{-3} M: NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, D-glucose 11.1, $CaCl_2$ 2.5) pre-gassed with 95% O_2/5% CO_2, at room temperature.

The collection of cadaveric tissue did not require ASPA authority, and the ASPA Schedule 1 method of killing was conducted within Queen Mary University of London, a UK Home Office licensed establishment (Ref XEDA0E7B1). The protocol was approved by the Animal welfare ethical review board for Queen Mary University of London and those involved were trained and signed off as competent by the Named Training and Competency Officer and Named Animal Care and Welfare Officer.

2.2. Functional experiments

Four adjacent muscle strips (~4x~15 mm) were cut parallel to circular muscle fibres from the forestomach and gastric antrum (respectively ~3 mm from the apex of the stomach and pyloric sphincter) and were used immediately. These were mounted in 10 ml tissue baths between platinum wire electrodes (1 mm diameter, 15 mm length, 10 mm apart) and isometric force transducers (MLT201/D, AD Instruments, UK) and bathed in Krebs-Henseleit solution, gassed with 95% O_2/5% CO_2 and maintained at 37 °C. Changes in muscle tone were recorded (milliNewtons; mN) using AcqKnowledge v3.8.1 data acquisition system (BIOPAC Systems, USA) on personal computers (Dell, www.dell.com/uk). Data were recorded using a sampling frequency of 5 samples per second as this provided a good resolution of recordings. The BIOPAC hardware was set to x200 gain, low pass filters to 10 Hz and 5 kHz and high pass filter to direct current, filtering out background electrical interference. Electrodes were connected to STG2008 stimulators (Multi Channel Systems, Germany). After 15min tissues were stretched by 10 mN and equilibrated for 1 h with renewal of Krebs-Henseleit solution every 20 min. Thereafter, the strips were treated with a test substance or vehicle for 30 min followed by construction of cumulative concentration-response curves for AVP or OT.

Electrical Field Stimulation (EFS) was applied as pulses of 5 Hz frequency, 0.5 ms width for 10 s/min at voltage 10% greater than that required to elicit maximal contractions (a pilot frequency-response experiment [50 pulses at 1–20 Hz] with two stomachs found that 5 Hz evoked submaximal contractions, respectively ~50% and ~30% of that elicited by 20 Hz and carbachol 10^{-4} M). After muscle tone and EFS-evoked contractions were stable for at least 30 min, test substances were applied for 30 min followed by construction of cumulative concentration-response curves for AVP or OT ($\log_{10}$-unit increments with 15 min intervals or when effect of previous concentration had peaked), with one curve constructed per tissue. Treatments were randomized between tissue baths and paired with time-matched vehicle-treated controls.

After completing an experiment, each muscle strip was challenged with carbachol, (10^{-4} M) to estimate maximal contractile capacity.

2.3. Data analysis

Spontaneous contractions (Fig. 1A) were quantified using custom software (https://github.com/agharibans/GISMCA) measuring change in baseline muscle tone (mN), amplitude of contractions (mN), number of contractions/minute (c/min) during steady-state, total contraction time (s), time for contractions to peak and decay (s), rates for contraction development and decay (mN/s) and areas under the contraction wave and during the development and decay phases (mN.s) (Fig. 1B). Measurements were visualized as radar plots using OriginPro 2020 (OriginLab Corporation, USA).

Agonist-induced increases in baseline muscle tone were expressed as percentage of carbachol-evoked contraction, whereas changes in spontaneous contraction amplitude and frequency were expressed relative to baseline values. For experiments with EFS, the effects of agonists were quantified as percentage change in amplitude of EFS-evoked contractions immediately before first addition.

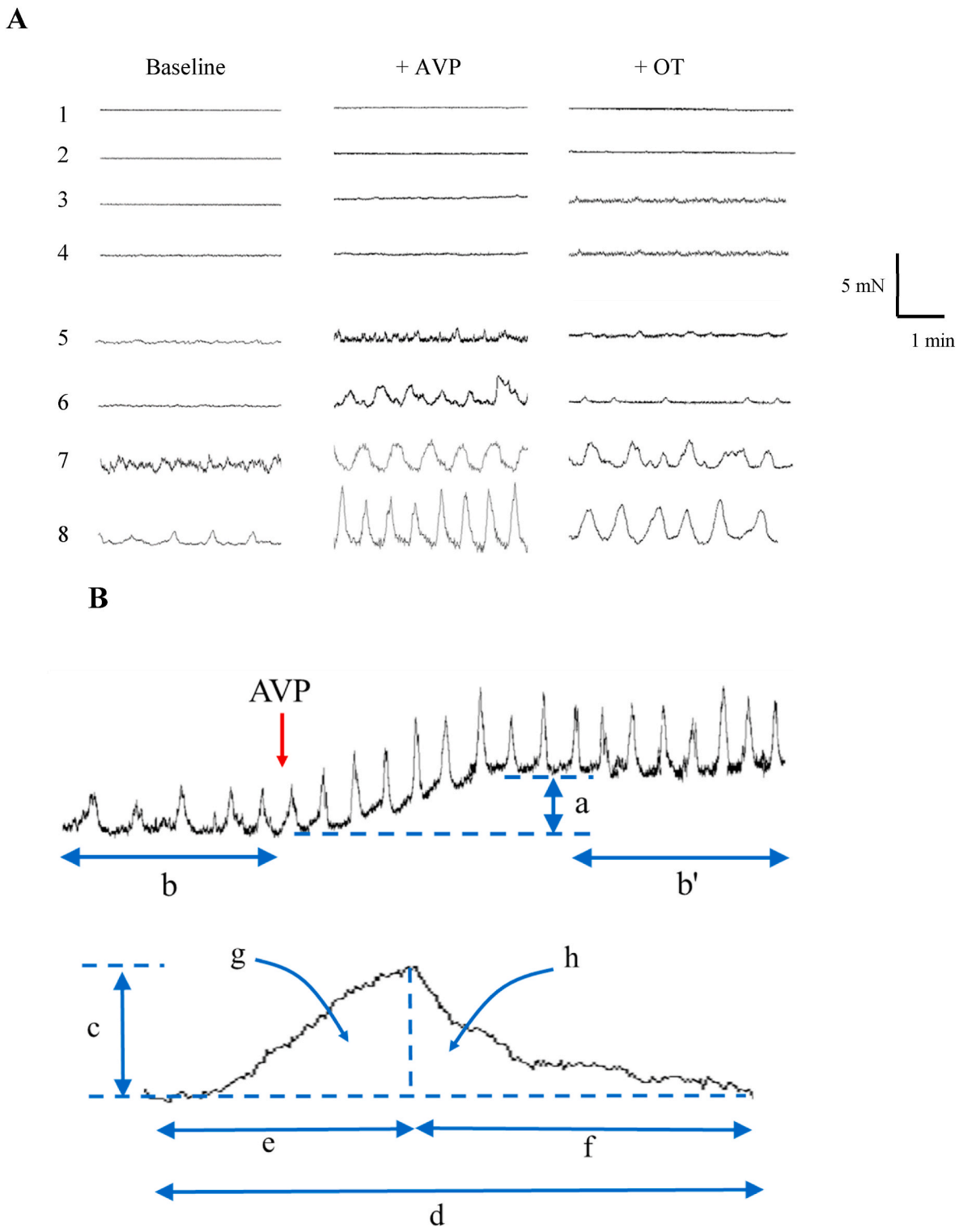


Fig. 1. Effects of AVP and OT on baseline motility of circular muscle strips cut from the rat forestomach and gastric antrum. (A) Representative recordings of motility for muscle strips in the absence (baseline) and presence of a maximal concentration of AVP (10^{-7} M) and OT (10^{-6} M). The muscle strips are numbered from nearest the forestomach apex (1), then as cut sequentially further down the forestomach (2–4), and from the antrum nearest the pyloric sphincter (8) then sequentially further up the antrum (5–7). (B) Spontaneous contractions of rat gastric antrum and change in a = muscle tone (mN) and frequency of contractions (number of contractions/minute; c.p.m.), usually measured over 5 min, b = before and b' = after application of an agonist (e.g AVP). Features measured of a given contraction wave, c = Contraction amplitude (mN), d = Total contraction time (s), e and f = Time for contraction to peak and decay (s), g and h = Area under the curve for contraction to peak and decay (mN.s). g and h = Total area under curve (mN/s), mean change in c over e and, h over f = Rates of contraction development and decay (mN/s). (C) Comparison between the different features of the spontaneous contraction waveform of a randomly selected forestomach strip and the distal-most strip of antrum, expressed as a radar plot. Also shown is the maximal increase in muscle tone caused by carbachol (10^{-4} M). Data are given as mean ± S.E.M, n = 12. (D) Effect of TTX (10^{-6} M), atropine (10^{-6} M), L-NAME (3×10^{-4} M) on spontaneous contractions recorded from the distal-most antrum muscle strip (number 8); n = 6.

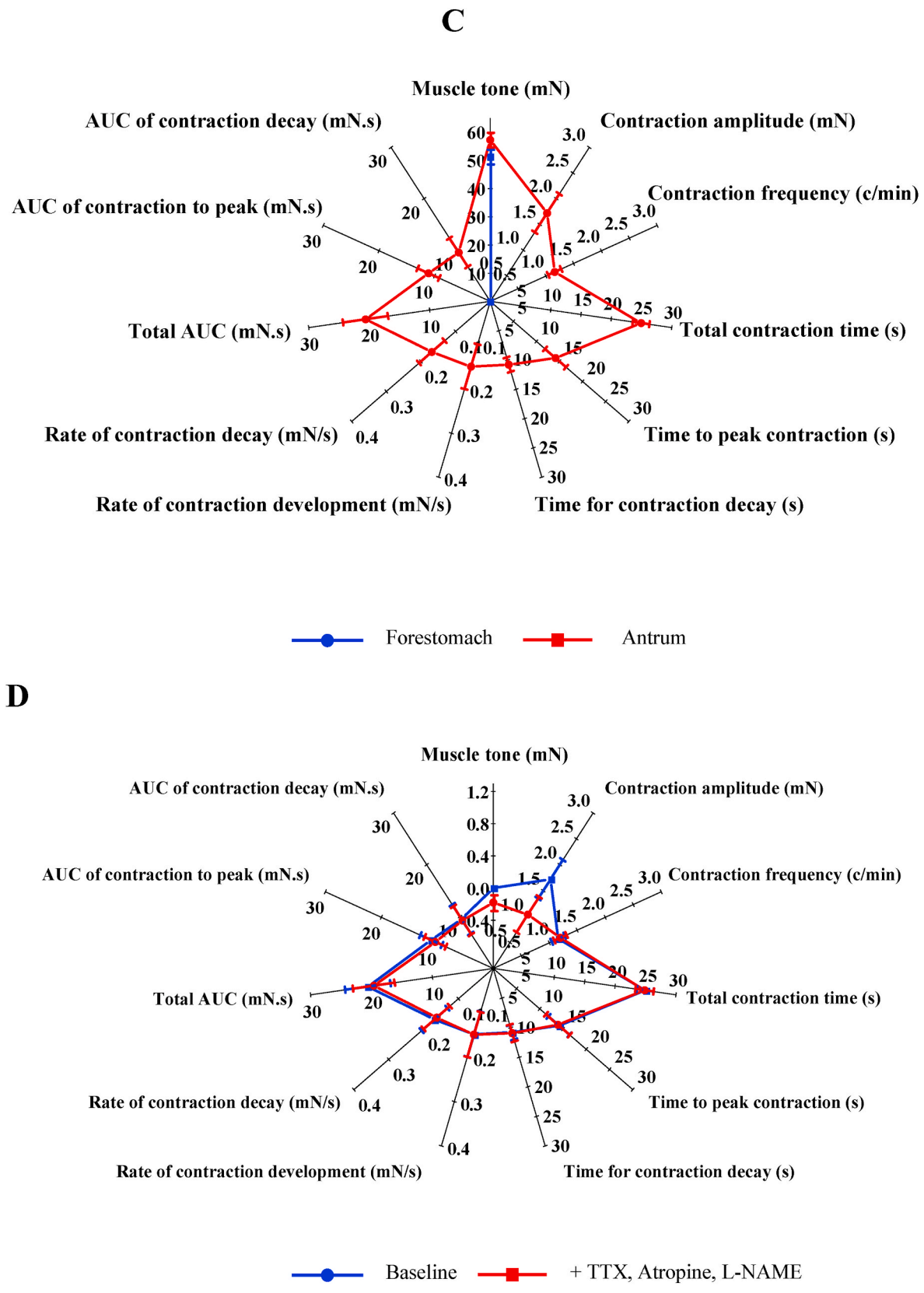


Fig. 1. (*continued*).

Individual agonist concentration-response curves were fitted by non-linear regression using a four-parameter logistic Hill equation using GraphPad PRISM 5.0 for Windows (Graph-Pad Software, La Jolla, CA, USA) as described previously (Makwana et al., 2022). Ratios for increased agonist pEC_{50} (negative $\log_{10}$ of agonist molar concentration eliciting 50% maximal effect) in the presence of increasing concentrations of antagonist were analyzed using Schild plots, determining the negative $\log_{10}$ of the equilibrium dissociation constant (pK_B) of the

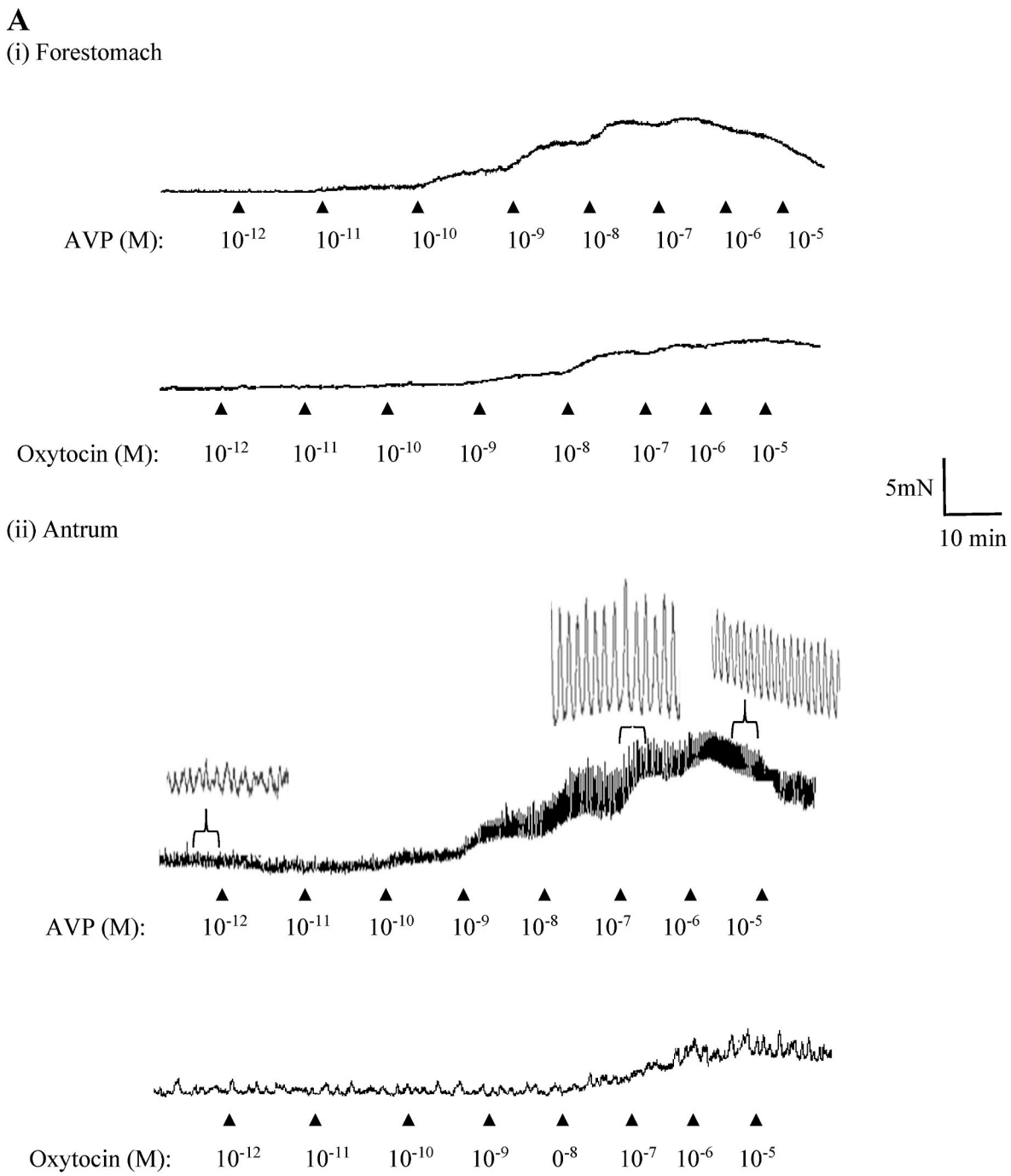
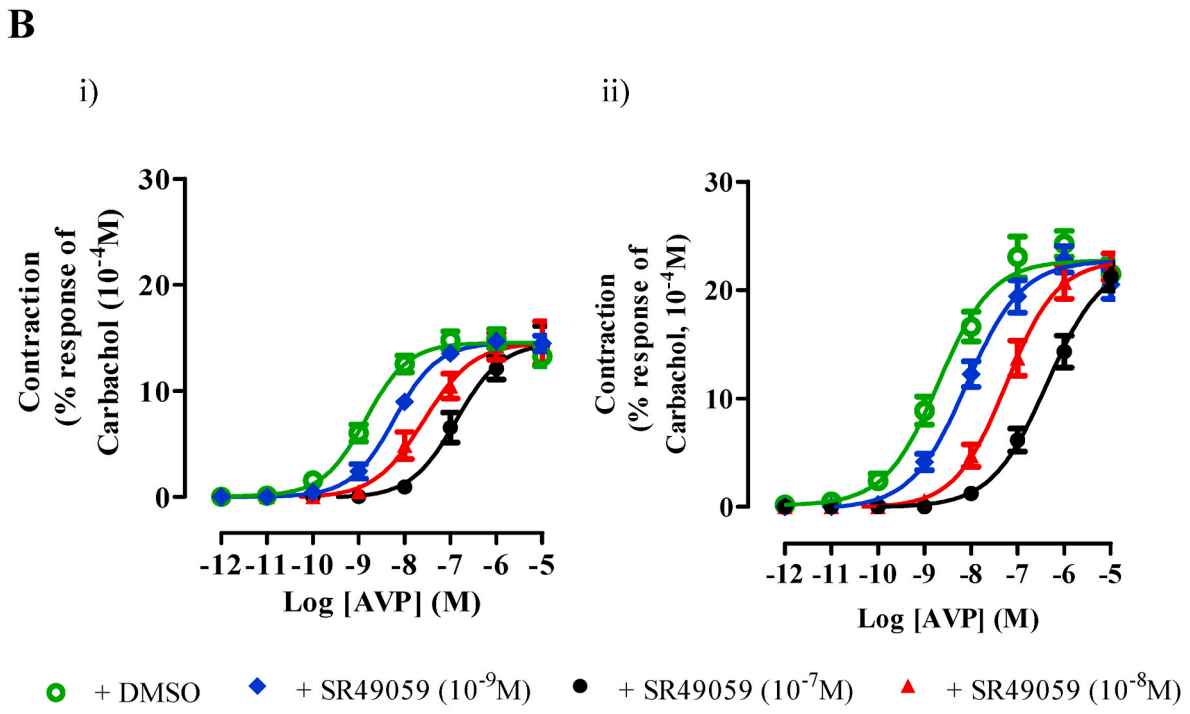


Fig. 2. (A) Effects of AVP and OT on muscle tone and spontaneous contractions of rat forestomach and distal gastric antrum circular muscle strips. Scale bar applies to all four concentration response recordings, not magnified parts (B) AVP- and (C) OT-induced increase in muscle tone of randomly selected (i) forestomach and (ii) distal antrum circular muscle strips, after 30min incubation with, respectively, SR49059 and L371257 or vehicle (DMSO 0.1%v/v). (D) AVP- and (E) OT-induced increase in muscle tone of randomly selected (i) forestomach and (ii) distal antrum circular muscle strips after 30 min incubation with a combination of tetrodotoxin (10^{-6} M), atropine (10^{-6} M) and L-NAME (3×10^{-4} M) or their vehicle (DMSO and water 0.5%v/v). Data are mean ± S.E.M., n = 6 (B and C) and n = 5 (D and E). Scale bar correct for each complete record shown.

antagonist-receptor complex.

2.4. Chemicals used

Atropine sulphate, carbachol (carbamylcholine chloride), L-NAME (Nω-nitro-L-arginine methyl ester hydrochloride), TTX (tetrodotoxin) and salts for Krebs-Henseleit solution (Sigma-Aldrich, Gillingham, UK). AVP ([Arg^8]-vasopressin), OT, SR49059 (V_{1A} antagonist; Serradeil-Le Gal et al., 1993) and L371,257 (oxytocin receptor antagonist; Griffante et al., 2005) (Tocris Biosciences, Abingdon, UK). Ascorbic acid, AVP, carbachol, L-NAME, oxytocin and TTX were dissolved in water. Compounds were dissolved in 100% dimethyl sulphoxide (DMSO). Total volume of solvents added to the tissue baths did not exceed 1% bath volume.



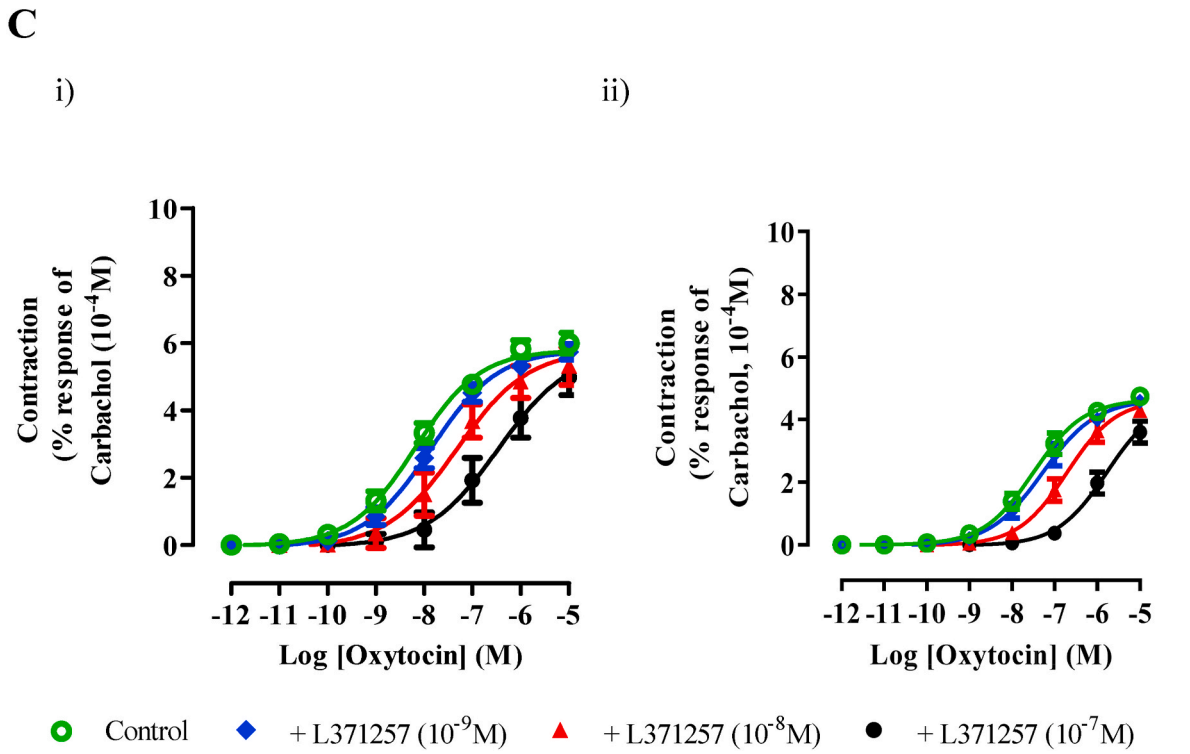


Fig. 2. (*continued*).

2.5. Statistical analysis

Data were quantified as mean ± standard error of the mean (S.E.M). Paired or unpaired Student's *t*-tests compared individual means of data from a given stomach or between stomachs from different animals, respectively. Shifts of agonist concentration-response curves by a test ligand were compared by one-way ANOVA followed by Dunnett's *post hoc* test for multiple comparisons. $P < 0.05$ represented statistical significance. *n* values represent number of animals. Only one muscle strip was used per drug treatment from a given animal.

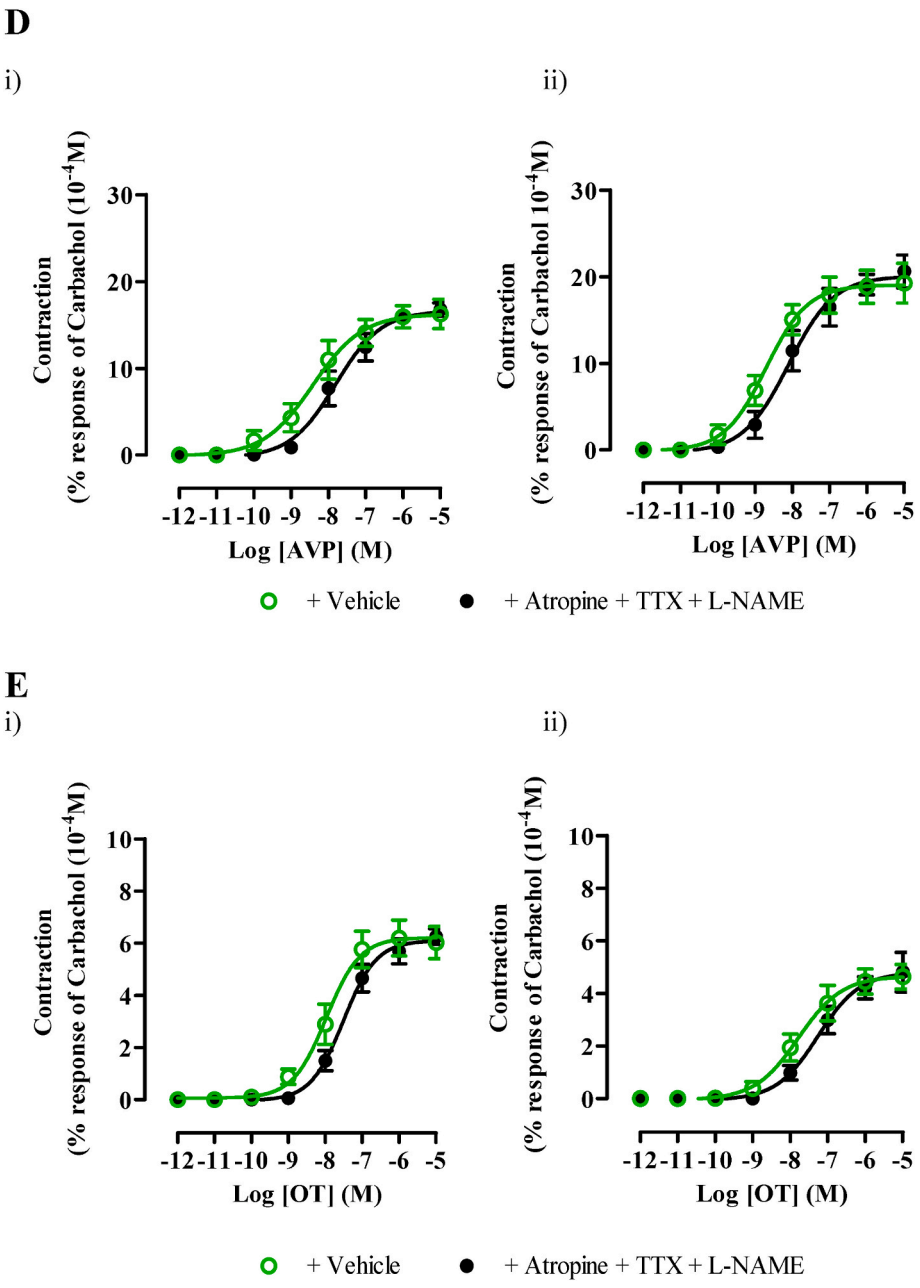


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Table 1
Threshold concentration, potency (pEC_{50}) and maximal effect (E_{max}) of AVP and OT, for increasing muscle tone, amplitude and frequency of spontaneous contractions of a randomly selected forestomach and distal-most antrum circular muscle strip of rat stomach.

	Forestomach						Distal-most antrum					
	AVP			OT			AVP			OT		
Effect on	Threshold (M)	pEC_{50}	E_{max} (%)	Threshold (M)	pEC_{50}	E_{max} (%)	Threshold (M)	pEC_{50}	E_{max} (%)	Threshold (M)	pEC_{50}	E_{max} (%)
Tone	10^{-11}-10^{-10}	8.9 ± 0.1	14.6 ± 0.2	10^{-10}-10^{-9}	8.2 ± 0.1	5.8 ± 0.1	10^{-11}-10^{-10}	8.7 ± 0.1	22.7 ± 0.4	10^{-10}-10^{-9}	7.2 ± 0.1	4.6 ± 0.1
Amplitude	–	–	–	–	–	–	10^{-10}-10^{-9}	8.3 ± 1.6	331.6 ± 52.4	10^{-8}-10^{-7}	6.5 ± 0.2	112.3 ± 36.7
Frequency	–	–	–	–	–	–	10^{-10}-10^{-9}	8.7 ± 1.0	42.2 ± 10.2	10^{-7}-10^{-6}	5.9 ± 0.9	18.1 ± 3.7

Data are mean ± S.E.M. n = 5–6.

Table 2
Schild slopes and pK_B values of SR49059 and L371257 for antagonism of respectively, AVP- and OT-induced increase in muscle tone of rat forestomach and antrum circular muscle.

		SR49059 *vs* AVP			L371257 *vs* OT		
Region	Effect on	Schild slope	pK_B	True pK_B (slope = 1.0)	Schild slope	pK_B	True pK_B (slope = 1.0)
Forestomach	**Tone**	1.0 ± 0.0 (1–1.1)	9.5 ± 0.2 (9.2–9.8)	9.4 ± 0.1 (9.3–9.5)	0.8 ± 0.1 (0.6–1.1)	9.0 ± 0.1 (8.9–9.3)	8.9 ± 0.0 (8.8–8.9)
Antrum	**Tone**	1.2 ± 0.1 (1.0–1.1)	9.5 ± 0.1 (9.4–9.6)	9.4 ± 0.1 (9.3–9.4)	0.9 ± 0.1 (0.8–1.1)	8.8 ± 0.1 (8.7–8.9)	8.8 ± 0.1 (8.7–8.8)

Data are mean ± S.E.M n = 6.

Table 3
Potency (pEC_{50}) and tissue maximal response (E_{max}) of (A) AVP and (B) OT for the increase in muscle tone of randomly selected rat forestomach and antrum circular muscle strips in the absence and presence of blockade of neurogenic, cholinergic and nitrergic activity.

A

Forestomach				Antrum			
Control		+ TTX + Atropine + L-NAME		Control		+ TTX + Atropine + L-NAME	
pEC_{50}	Emax (%)	pEC_{50}	Emax (%)	pEC_{50}	Emax (%)	pEC_{50}	Emax (%)
8.4 ± 0.1	16.3 ± 0.5	7.8 ± 0.1	16.6 ± 0.5	8.7 ± 0.1	19.1 ± 0.5	8.1 ± 0.1	20.1 ± 0.6

B

Forestomach				Antrum			
Control		+ TTX + Atropine + L-NAME		Control		+ TTX + Atropine + L-NAME	
pEC_{50}	Emax (%)	pEC_{50}	Emax (%)	pEC_{50}	Emax (%)	pEC_{50}	Emax (%)
8.0 ± 0.1	6.2 ± 0.2	7.5 ± 0.1	6.1 ± 0.1	7.9 ± 0.1	4.7 ± 0.2	7.3 ± 0.1	4.8 ± 0.2

Blockade of neurogenic, cholinergic (muscarinic) and nitrergic transmission was achieved by simultaneous incubation with TTX (10^{-6} M), atropine (10^{-6} M) and L-NAME (3×10^{-4} M), respectively, 30 min before examining (A) AVP and (B) OT. Data are mean ± S.E.M. n = 5.

3. Results

3.1. Spontaneous muscle contractions

Spontaneous contractions were not observed in the forestomach but were observed in the antrum (Fig. 1A). However, within the antrum regular and rhythmic contractions were observed only in the two prepyloric strips. Further, the contractions of the most distal strip were greater in amplitude and uniformly rhythmic, enabling the contraction parameters to be measured (e.g., amplitude 1.7 ± 0.4 mN; frequency 1.2 ± 0.1 contractions/min, n = 12) for display as a radar plot (Fig. 1C). For these experiments and in each muscle strip from each region the maximum contraction to carbachol (10^{-4} M) was similar; e.g., proximal forestomach (51.3 ± 2.6 mN) and most distal antrum (57.3 ± 2.6 mN; n = 12 each).

Compared to time-matched controls, a combination of TTX (10^{-6} M), atropine (10^{-6} M) and L-NAME (3×10^{-4} M) caused small tone reductions in the forestomach and antrum (respectively, by 0.3 ± 0.1 mN; P = 0.0009 and 0.4 ± 0.2 mN; P = 0.0012, n = 6 both). The ligands also tended to reduce the amplitude of the spontaneous contractions, but this effect did not reach statistical significance (n = 6, P = 0.12) (see Fig. 1D for the distal-most antrum).

3.2. Effects of arginine vasopressin and oxytocin on muscle tone

In both stomach regions, the application of AVP and OT caused muscle contraction, which were increased in a concentration-dependent manner but with a greater maximum response in the antrum for AVP; similar potencies were observed in both stomach regions for each hormone (Fig. 2). Overall, OT was ~0.5 $\log_{10}$-unit less potent (P = 0.001) than AVP (Table 1).

SR49059 (V_{1A} receptor antagonist [13]) and L371257 (oxytocin receptor antagonist [14]) did not affect spontaneous contractions of the antrum, nor baseline muscle tone (data not shown), but antagonized the ability of AVP and OT to increase muscle tone (Fig. 2B and C; Table 2). The presence of atropine (10^{-6} M), TTX (10^{-6} M) and L-NAME (3×10^{-4} M) (Fig. 2D and E, Table 3) resulted in a small reduction (by ~0.5 $\log_{10}$-units) in the increase in muscle tone in response to AVP and OT (P = 0.006).

3.3. Effects of arginine-vasopressin and oxytocin on spontaneous muscle contractions

OT and AVP did not alter the quiescent state of any forestomach circular muscle strip (Figs. 1A and 2A). In the antrum application of both hormones caused the spontaneous contractions to become more regular in amplitude and frequency (Figs. 1A and 2A). For the distal antrum, where changes in spontaneous contractions were quantified, OT or AVP were found to increase the amplitude and frequency of spontaneous contractions in a concentration-dependent manner (Fig. 3A). In this activity, OT was ~2 $\log_{10}$-units less potent and efficacious (~35%) compared to AVP and did not significantly augment contraction frequency (Table 1). Examination of the radar plot (Fig. 3B) showed that in the prepyloric region of the antrum, the maximally-effective concentration of AVP increased the frequency of contractions by respectively increasing and decreasing the rate and duration of contraction development and decay. Coupled to the increased amplitude of contraction, this resulted in similar changes in 'area under the curve' for each phase of contraction. Application of OT generated broadly similar effects but was less efficacious in terms of increasing contraction amplitude and frequency.

3.4. Electrical field stimulation-evoked contractions and effect of AVP and oxytocin

In both stomach regions EFS elicited a small relaxation during stimulation followed by a large 'rebound' contraction on termination. During the first 20 min of stimulation, the amplitude of the relaxations diminished as the muscle tone decreased from 10 mN to ~5 mN whereas the amplitude of the rebound contractions gradually increased and continued to increase for up to 2 h before stabilizing for 3–4 h.

EFS-evoked contractions of forestomach appeared to have a

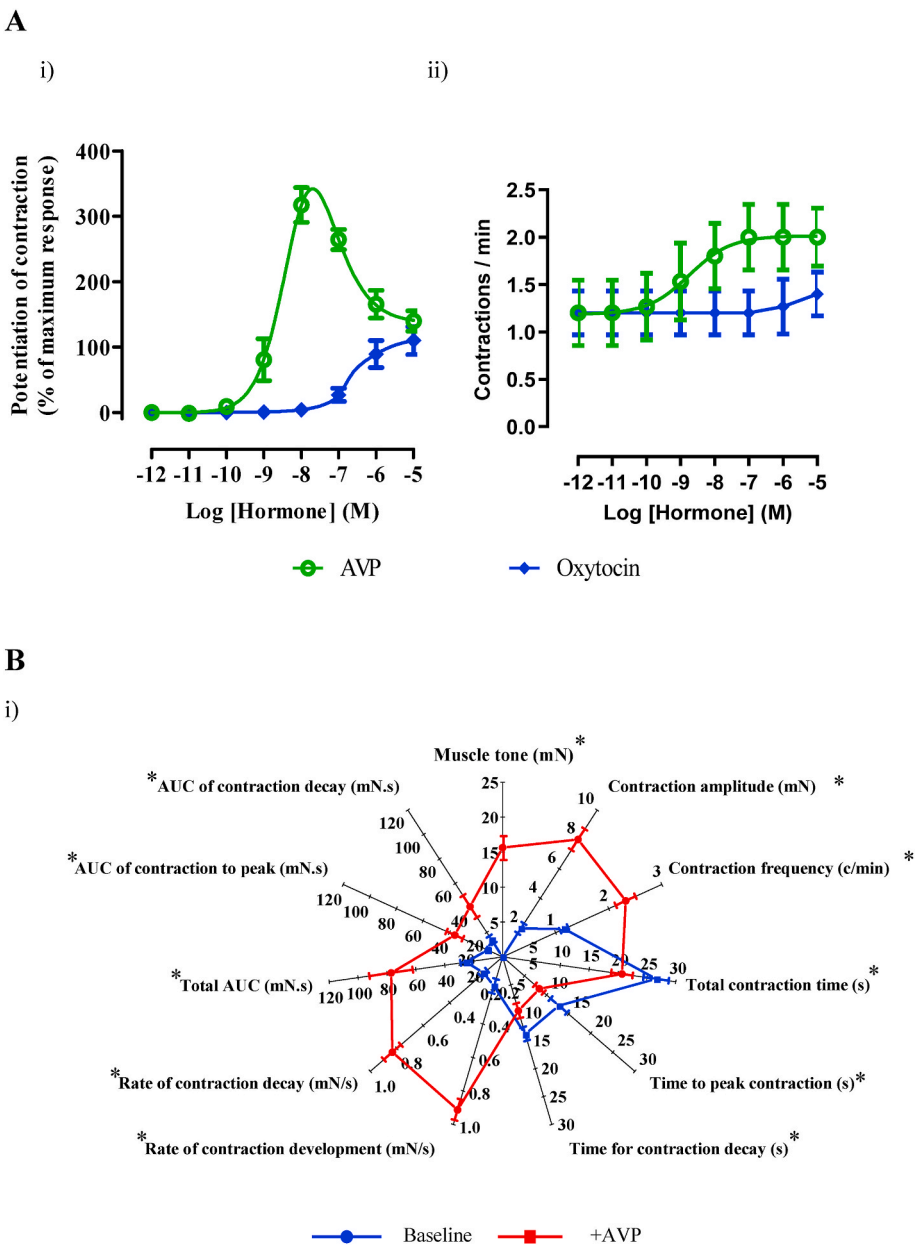

Fig. 3. (A) Effects of different concentrations of AVP and OT on (i) amplitude and (ii) frequency of spontaneous contractions of rat distal gastric antrum circular muscle strips. (B) Comparison between the various features of the spontaneous contraction waveform of rat antrum by (i) AVP (10^{-8} M) and (ii) OT (10^{-7} M). Data are mean ± S.E.M. n = 6. * indicates $P < 0.05$.

"rectangular hyperbolic" shape i.e., developing with a faster rate initially and then approaching a plateau before decaying quickly to baseline levels. In comparison, the EFS-evoked contractions of the antrum developed and decayed relatively slowly than those of the forestomach (Fig. 4). At equilibrium the amplitudes of rebound contractions of randomly selected forestomach and antrum strips were, respectively, 19.4 ± 1.6 mN and 18.1 ± 2.4 mN (n = 8 both). These contractions were consistently abolished by atropine (10^{-6} M, n = 5) or TTX (10^{-6} M, n = 5). AVP and OT each partially inhibited the contractions and increased muscle tone (Fig. 4), with AVP more potent and efficacious, particularly in forestomach. L-NAME (3×10^{-4} M) did not alter AVP-induced inhibition of EFS-evoked contractions (Table 4).

4. Discussion

The present study found that the circular muscle of the rat gastric antrum spontaneously contracted in an irregular or regular manner, depending on proximity to the pylorus. Only in muscle strips cut from nearest the pyloric sphincter were the amplitude and frequency of contractions found to be regular. In this prepyloric region contractions occurred at 1.2/min, lower than that reported by others, but consistent with the findings in similar experiments with mouse gastric antrum (Guo et al., 2003; Makwana et al., 2021). These contractions were 'myogenic', that is they were not significantly affected by the presence of TTX, atropine and L-NAME. Although not studied here, comparable studies with mouse, guinea-pig and human stomach (e.g., Hirst and Edwards, 2006; Rhee et al., 2011; Worth et al., 2015; Straface et al., 2022) suggest that spontaneous myogenic contractions are generated by ICC

ii)

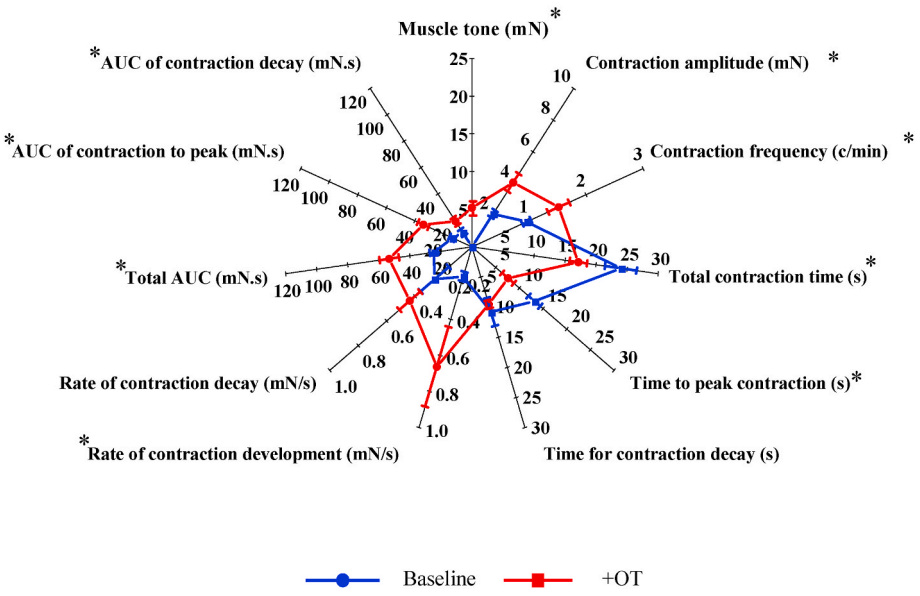

Fig. 3. (*continued*).

depolarization. The absence of meaningful myogenic activity in the rat forestomach is consistent with sparse existence of ICCs within this region, generating only small-amplitude, or 'noise-like' electrical events (Christensen et al., 1992; Kito et al., 2009).

Comparisons between experiments with rat and human stomach are complicated by differences in gross anatomy (e.g., compared with human, a lower entry point of the oesphagus into the stomach, with a large 'forestomach' above this point of entry) and in functions between the different regions. For the rat and other rodents these may have evolved to enable consumption of large volumes of food and facilitate a 'steady state' of gastric emptying and digestion, losing the ability to vomit and using the forestomach as a food store (Gärtner, 2001; Sanger et al., 2011). Thus, the large forestomach (~2/3rd total stomach volume) accommodates large volumes of food without increasing intragastric pressure or gastric emptying rate, retaining food for up to 2 h before moving it to the antrum (Gärtner, 2001). In humans, accommodation to food ingestion occurs over a much shorter time in the smaller fundus and upper corpus before propulsion into the distal corpus and antrum; for solid food an approximate 30–60 min 'lag phase' exists between ingestion and the beginning of gastric emptying (Siegel et al., 1998; Hellström et al., 2006). Spontaneous electrical slow waves are not detected in human gastric fundus (O'Grady et al., 2021), an observation comparable with the absence of myogenic contractions in rat forestomach. However, spontaneous contractions (associated with ICC activity; Rhee et al., 2011; Straface et al., 2022) were observed in the more distal human proximal (fundus-corpus border) and distal (proximal antrum) stomach, at a frequency of approximately 3. min^{-1} (Makwana et al., 2022; Straface et al., 2022); see also Rhee et al. (2011), where 5–8.min^{-1} has been reported. Compared with the proximal stomach, in which the times to peak contraction and recovery were similar, contractions of the distal human stomach were larger, with a relatively fast development to peak contraction followed by slower recovery (Makwana et al., 2022). Notably, these characteristics differ from those found in the present experiments with rat gastric antrum. Thus, in the rat, contractions of the antrum were irregular, except at the prepyloric region where the times to peak contraction and recovery were similar.

The reason for the change in contraction characteristics, from irregular to regular, within different regions of the rat gastric antrum is not clear. In human stomach, slow waves of electrical activity propagate aborally as 'ring wavefronts', which abruptly increase in velocity and amplitude at the prepyloric antrum (contributing to forceful terminal antral contractions), before terminating thereafter; it was speculated by the authors that this acceleration may be related to enhanced connectivity between the ICC (e.g., enhanced gap-junction coupling; Berry et al., 2016). Perhaps, in the rat stomach, coupling between the ICC and/or between the ICC and muscle, and entrainment of distal ICCs is generally less efficient than in human, so myogenic contractions are regular only in the prepyloric antrum. Further experiments are needed, measuring muscle contraction and electrical activity together. It would also be of interest to compare the present data with an analysis of the myogenic contractions in the intact rat isolated stomach (e.g., in the mouse stomach myogenic contractions are regulated by non-neuronal acetylcholine; Cai et al., 2022).

In both rat stomach regions, AVP and OT caused tonic contraction (via, respectively V_{1A} and OT receptors), an action sensitive at least partially, to blockade of neurotransmission. Notably, others (Qin et al., 2009a) reported abolition of AVP-induced contractions of rat stomach by TTX; the reason for the marked difference in magnitude of the effect of TTX between these and the present experiments is not clear.

In the antrum, but not the quiescent forestomach, AVP and OT regularized and/or amplified the myogenic contractions; OT was less potent than AVP. Interestingly, AVP and OT also partially reduced neuronally-mediated contractions; the mechanism is unclear but unlikely to be mediated by enteric nitric oxide. These findings have similarities and differences with those previously reported for the stomach of rat and other species. For example, in the rat antrum, the excitatory activity of OT is consistent with the findings of Qin et al. (2009b) but differs from the ability of OT to inhibit spontaneous contractions of guinea-pig gastric antrum (Duridanova et al., 1997). In conscious rats, intraperitoneal administration of the AVP analogue terlipressin, increased the frequency of gastric slow waves (Nowak et al., 2004) In rabbits, intravenous OT or AVP dose dependently increased stomach pressure via receptors blocked by atosiban (Li et al., 2007), but at doses in which selectivity for the OT and/or the AVP receptors was unclear. In cat stomach, an emetic species, AVP but not OT contracted the longitudinal muscle and increased the amplitude but not frequency of spontaneous contractions; atropine reduced the AVP-induced contractions

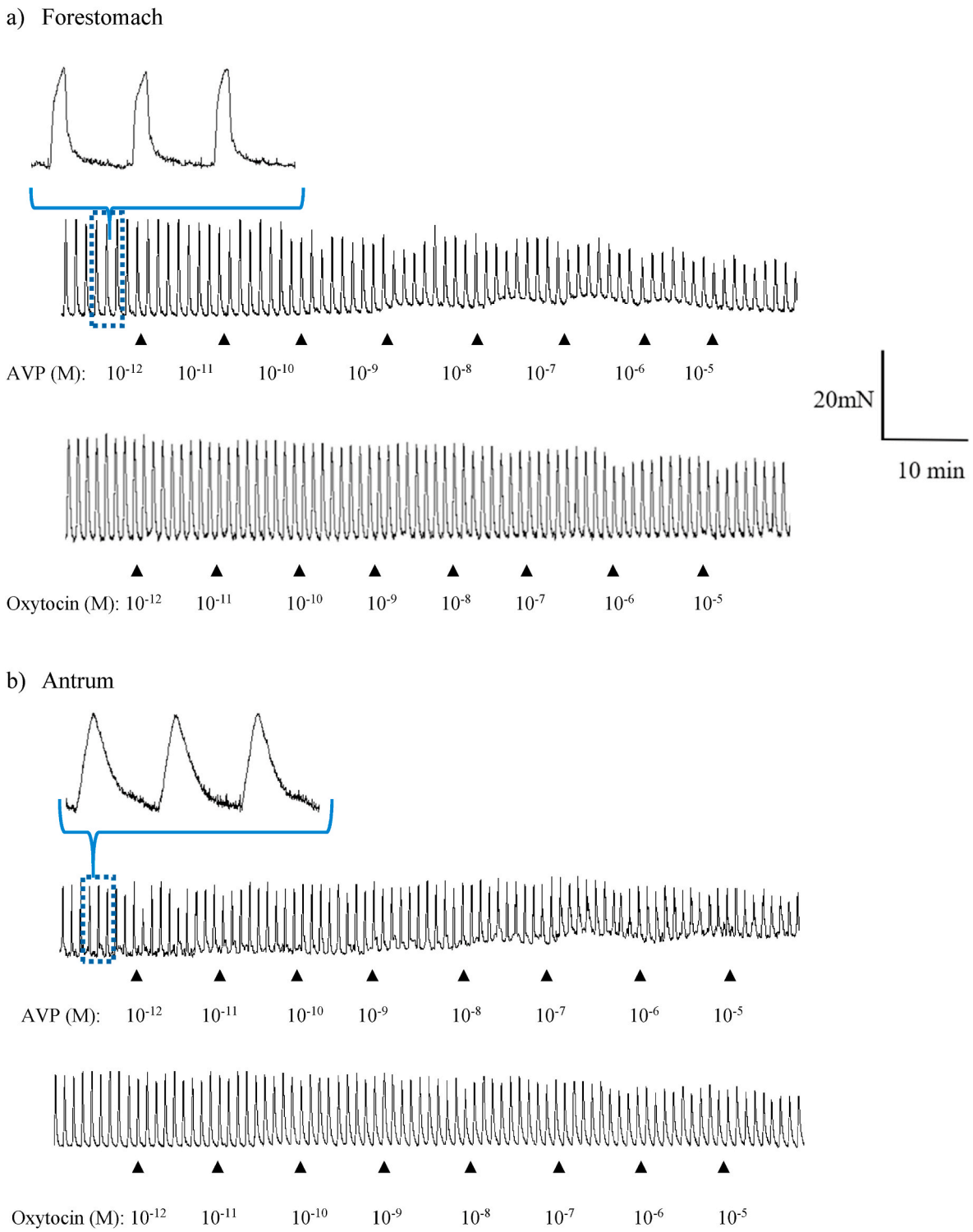

Fig. 4. Electrical field stimulation (EFS)-evoked isometric contractions of randomly selected circular muscle strips of the rat isolated (a) forestomach and (b) antrum, in the absence and presence of increasing concentrations of AVP and oxytocin. Scale bar applies to all concentration response recordings, not magnified parts. EFS parameters: Trains of pulses of 5 Hz frequency for 10 s every 1 min, 0.5 ms duration and 110% supramaximal voltage. c) Concentration response curves for the inhibition of the EFS-evoked contractions by AVP and oxytocin. Each curve was fitted by non-linear regression analysis. Each symbol represents the mean value of inhibition of contractions expressed as a percentage reduction of the amplitude of contractions measured immediately before the addition of the hormone to the organ bath. Vertical lines indicate s.e.m. n = 5.

and inhibited the spontaneous contractions (Mirčić et al., 1998). In dogs, also an emetic species, intravenous administration of AVP caused ectopic gastric electrical dysrhythmia (Du et al., 2016). Finally, in human stomach, AVP and OT, also through V_{1A} and OT receptors, respectively, caused muscle contraction and increased the amplitude, frequency and rate of spontaneous contractions of the antrum and the proximal stomach (corpus-antrum); these actions were unaffected by TTX or atropine and further, AVP and OT had no effects on

c)

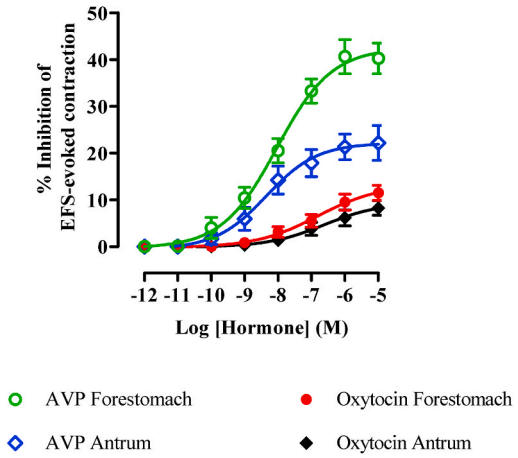

Fig. 4. (*continued*).

Table 4
Potency (pEC_{50}) and maximal percentage inhibition of EFS-evoked contractions of a randomly selected forestomach and antrum circular muscle strip of rat isolated stomach by AVP in the absence and presence of L-NAME (3×10^{-4} M).

	Forestomach		Antrum	
	pEC_{50}	% inhibition	pEC_{50}	% inhibition
AVP	8.0 ± 0.2	42.5 ± 2.7	8.4 ± 0.1	22.2 ± 1.0
AVP + L-NAME	8.2 ± 0.2	44.2 ± 1.3	8.3 ± 0.2	22.6 ± 1.5

Data are mean ± S.E.M. (n = 5).

electrically-evoked, neuronally-mediated contractions (Makwana et al., 2022). In the human stomach (Makwana et al., 2022), the potency of OT was greater than in the present experiments with rat stomach.

In summary, the results from the present experiments with rat stomach are consistent with our original hypothesis, that in a non-vomiting species, the characteristics of gastric myogenic contractions would differ from those in human stomach. Clearly, an important limitation is that only a limited number of different species have been examined and similar experiments are now required using other species, both rodent and other mammals with an ability to vomit. Interestingly, AVP and OT were found to exert broadly similar excitatory activity in rat stomach as they do in human stomach, with the potency of OT less than for AVP in both species. This similarity was at first, surprising since OT is released instead of AVP by stimuli which in a vomiting species would be emetic and release AVP not OT. In rodents, the release of OT by an 'emetic' stimulus may cause taste aversion or anorexia by acting centrally (Verbalis et al., 1986; Arase et al., 2018), whereas release of AVP may play a role in stress-induced anorexia (Langhans et al., 1991) and suppression of appetite during high plasma osmolality (Ikemura et al., 2004). Perhaps these different actions are somehow related to the greater activity of AVP in rat stomach compared with OT, but further experiments are needed to investigate any such connection. Finally, it is important to note that the present experiments demonstrate robust excitatory actions of OT and AVP, yet *in vivo*, OT inhibited rat gastric emptying (Wu et al., 2002) and in anesthetized rats OT caused transient inhibition of gastric motility, followed by excitation (Qin et al., 2009b). Similarly, intraperitoneal administration of AVP can reduce rat gastric emptying by a mechanism not blocked by hepatic vagotomy (Langhans et al., 1991). The reasons for the different observations made *in vitro* and *in vivo* are not clear although Wu et al. (2002) found that *in vivo*, the actions of OT were in part mediated by the release of cholecystokinin. A similar conundrum exists for human stomach (Makwana et al., 2022). Possible explanations include the possibility that the marked excitatory actions of OT/AVP destroy entrainment between the prepyloric antrum and the less well coordinated contractions originating in the corpus, leading to 'collision' of slow waves and non-propulsive movements (with an additional ability of OT to contract the rat pyloric sphincter). Alternately, OT/AVP may stimulate vagal nerve mechanoreceptors (initiating a vago-vagal reflex inhibition of gastric motility; Qin et al., 2009b).

In conclusion, the characteristics of spontaneous myogenic contractions of the rat stomach are not the same as in human stomach, consistent with the different structures and functions of different regions of stomach between the two species, and arguably related to different feeding characteristics and/or inability or ability to vomit. Surprisingly, the actions of OT and AVP are broadly similar in both species, in spite of their different physiological roles.

Declaration of data transparency and availability

All authors had access to the study data and had reviewed and approved the final manuscript. The data and methods that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

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CRediT authorship contribution statement

Raj Makwana: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Gareth J. Sanger:** Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing.

Declaration of competing interest

RM declares no potential conflict of interest. GJS was in receipt of research funding from Takeda Pharmaceuticals and acts as scientific advisor for BYOMass, Nurix and Viwit Pharmaceuticals.

Data availability

Data will be made available on request.

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