

Clonidine and Dexmedetomidine Potently Inhibit Peristalsis in the Guinea Pig Ileum In Vitro

Michael K. Herbert, M.D., Susanne Roth-Goldbrunner, M.D.,* Peter Holzer, Ph.D.,† Norbert Roewer, M.D.‡*

Background: **Inhibition of intestinal peristalsis is a major side effect of drugs used for anesthesia or for analgesia and sedation of patients in the intensive care unit. This *in vitro* study examined the effect of clonidine and dexmedetomidine on intestinal peristalsis and analyzed some of their mechanisms of action.**

Methods: **In isolated segments of the guinea pig small intestine, peristalsis was triggered by a perfusion-induced rise of the intraluminal pressure. The peristaltic pressure threshold to elicit a peristaltic wave was used to quantify drug effects on peristalsis. Vehicle (Tyrode's solution), clonidine (10 nM–100 μM), or dexmedetomidine (0.1–100 nM) were added extraserosally to the organ bath. In other series of experiments, clonidine or dexmedetomidine was administered after pretreatment with yohimbine, prazosin, apamin, naloxone, or vehicle. Clonidine was also tested after blockade of NO synthase with L-NAME and in the presence of the inactive enantiomer D-NAME.**

Results: **Clonidine and dexmedetomidine concentration-dependently increased peristaltic pressure threshold and inhibited peristalsis (clonidine: EC_{50} = 19.6 μM; dexmedetomidine: EC_{50} = 12.0 nM). The inhibition caused by clonidine could be prevented by pretreatment with yohimbine, naloxone, and apamin, but not by prazosin, L-NAME, or D-NAME. Inhibition caused by dexmedetomidine was prevented by yohimbine only.**

Conclusions: **The results reveal that clonidine and, much more potently, dexmedetomidine inhibit peristalsis of the guinea pig ileum *in vitro*. The inhibition is caused by interaction with α_2 adrenoceptors and, in the case of clonidine, also involves activation of small conductance Ca^{2+}-activated potassium channels and endogenous opioidergic pathways.**

IMPAIRMENT of intestinal peristalsis by drugs used in anesthesia or in intensive care is a major side effect that has attracted little attention so far. Intestinal ileus may cause further problems in critically ill patients, such as bacterial overgrowth and translocation in the gastrointestinal tract, pulmonary infection, impairment of enteral nutrition, or dysfunction of the intestinal mucosal barrier due to villus atrophy.[1,2]

The α_2-adrenoceptor agonists clonidine and dexmedetomidine are used in anesthesia and intensive care due to their sedative, amnestic, analgesic, and anesthetic properties.[3–6] Furthermore, perioperative administration of clonidine and dexmedetomidine attenuates hyperadrenergic states[7–9] and thus provides hemodynamic stability in the face of stressful perioperative events.[5] Clonidine is the prototypical α_2 agonist and has been studied for more than three decades. Dexmedetomidine has a relatively high ratio of α_2/α_1 activity (1620:1 as compared with 220:1 for clonidine)[10] and therefore is considered a more specific agonist at the α_2 adrenoceptor.

Experimental studies in different animal species and humans[11–15] and clinical observations[16–18] suggest an inhibitory effect of clonidine on intestinal motility. Because of the lack of appropriate models, the effects of clonidine and dexmedetomidine on intestinal peristalsis have not yet been investigated in detail, and the mechanism of their inhibitory effect on peristalsis is unknown. Therefore, a preparation with isolated segments of the guinea pig small intestine *in vitro*[19] was used to record propulsive peristalsis in an organ bath and to explore and quantify drug effects on peristaltic motor activity. The present series of studies was designed to characterize the inhibitory effect of clonidine and dexmedetomidine on intestinal peristaltic motility and to shed some light on their mechanism of action.

Materials and Methods

Recording of Peristalsis

After obtaining approval from the Animal Care and Use Committee at the Regierung von Unterfranken in Wuerzburg, Germany, adult guinea pigs (BFA strain; Charles-River Wiga, Sulzfeld, Germany) of either sex weighing between 420 and 570 g were stunned and bled *via* the carotid arteries. The distal jejunum and ileum were excised, flushed of luminal contents, placed in Tyrode's solution at room temperature, and gassed with 95% O_2 and 5% CO_2 until required. The composition of the Tyrode's solution was as follows: 136.9 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 11.9 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, and 5.6 mM glucose. For studying peristalsis, the distal small intestine (at least 10 cm proximal to the ileocecal valve) was divided into segments, each being approximately 8–10 cm long. Five intestinal segments were set up in parallel in silanized glass organ baths containing 30 ml Tyrode's solution at 37°C. The system for eliciting and recording propulsive peristalsis has previously been described.[20] In brief, the oral end of the intestinal segment was tied to an inflow cannula, which permitted the continuous infusion of prewarmed Tyrode's solution at a flow rate of 0.5 ml/min (fig. 1A). The aboral end of the segment was attached to an intermediate tubing (ID, 4 mm) fixed with a T piece. One arm

* Staff Anesthesiologist, ‡ Professor and Chair of Anesthesiology, Department of Anesthesiology, University of Wuerzburg. † Professor of Neuropharmacology, Department of Experimental and Clinical Pharmacology, University of Graz, Austria.

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Address reprint requests to Dr. Herbert: Department of Anesthesiology, University of Wuerzburg, Josef-Schneider-Strasse 2, D-97080 Wuerzburg, Germany. Address electronic mail to: mherbert@anaesthesie.uni-wuerzburg.de. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

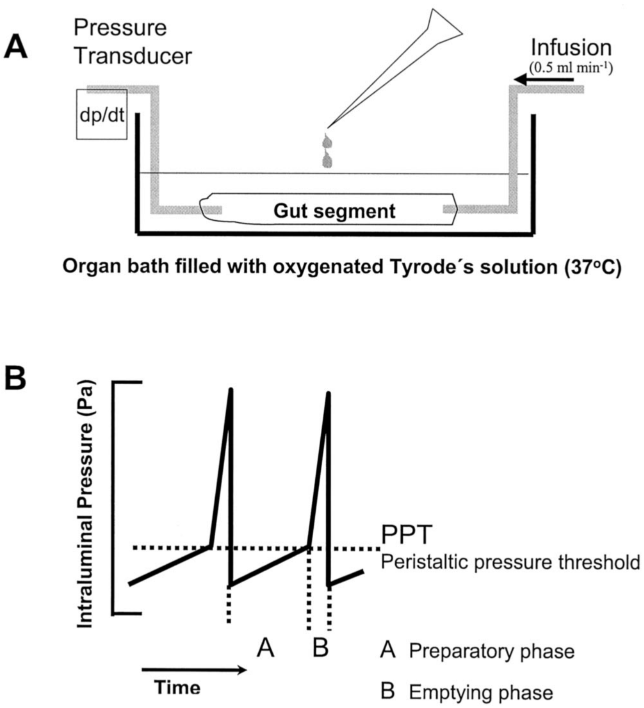

Fig. 1. (*A*) Schematic diagram of the organ bath arrangement for recording peristalsis in isolated segments of the guinea pig small intestine. (*B*) Schematic recording of peristaltic waves.

of the T piece was connected to a pressure transducer for recording the intraluminal pressure, and the other arm of the T piece was fitted with a vertical outlet tubing that ended 4 cm above the fluid level of the organ bath. This arrangement made emptying of the intestinal segment possible when peristaltic contractions raised the intraluminal pressure above 400 Pa. The records showed two distinct phases of intraluminal pressure changes (fig. 1B). During the preparatory phase, the intestine was gradually filled with fluid, and the intraluminal pressure rose slowly because the outlet tubing prevented the escape of fluid from the system. When the intraluminal pressure reached a threshold (peristaltic pressure threshold [PPT]; range, 37–68 Pa), an aborally moving wave of peristaltic contraction was triggered, and the emptying phase of peristalsis was initiated (fig. 1B). The wave of circular muscle contraction, measured as a spike-like increase in intraluminal pressure, propelled the intraluminal fluid to leave the system *via* the outlet tubing and thus caused partial emptying of the segment. The intraluminal pressure at the aboral end of the segments was measured with a pressure transducer whose signal was, *via* an analog/digital converter, fed into a personal computer and recorded simultaneously on a pen recorder.

Experimental Protocol

The preparations were allowed to equilibrate in the organ bath for a period of 30 min, during which they were kept in quiescent state. Thereafter, the bath fluid was renewed, and peristaltic motility was initiated by intraluminal perfusion of the segments. After basal peristaltic activity had been recorded for at least 30 min, the drugs to be tested were administered to the bath, *i.e.*, to the serosal surface of the intestinal segments, at volumes not exceeding 1% of the bath volume. All vehicle solutions used in this study were tested separately to ensure that they were devoid of any influence on peristaltic activity.

Each segment was exposed to only one drug concentration, and each concentration of dexmedetomidine (0.1–100 nM) and of clonidine (0.01–100 μM) was tested on six segments from six different guinea pigs or eight segments from eight guinea pigs, respectively. The peristaltic motor activity was recorded for 60 min after addition of the drug.

In separate experiments, some of the mechanisms mediating the inhibitory effect of clonidine and dexmedetomidine were investigated. Twenty minutes prior to the addition of either clonidine or dexmedetomidine, the following antagonists were administered to the organ bath, each in eight segments: (1) 1 μM yohimbine (antagonist at α_2 adrenoceptors) or 1 μM prazosin (antagonist at α_1 adrenoceptors); (2) 0.5 μM apamin (blocker of small conductance Ca^{2+}-activated potassium channels); (3) 0.5 μM naloxone (antagonist at opioid receptors); and in addition, in the case of clonidine, (4) the nitric oxide (NO) synthase inhibitor L-nitro-arginine methyl ester (300 μM, L-NAME) and its inactive enantiomer D-nitro-arginine methyl ester (300 μM, D-NAME). Thereafter, clonidine was administered at the concentration of 10 μM and dexmedetomidine at the concentration of 3 nM. The effect of clonidine and dexmedetomidine recorded in the presence of the antagonists was compared with that seen in the presence of vehicle (Tyrode's solution). In further experiments, first 3 nM dexmedetomidine was added to the segments, and after 20 min, the α_2-adrenoceptor antagonist yohimbine (1 μM) was administered to test the reversibility of the effect of dexmedetomidine on PPT.

Drugs and Solution

Clonidine was purchased from Tocris Cookson Ltd. (Bristol, United Kingdom), and dexmedetomidine was a gift from Abbott (Wiesbaden, Germany). All other chemicals were from commercial sources and of the highest purity available. The chemicals were dissolved in sterile water, and stock solutions were diluted with Tyrode's solution before use.

Evaluation of Results

Peristaltic pressure threshold was used to quantify drug effects on peristalsis. After regular peristaltic contractions had been recorded for at least 20 min, the PPT of the last peristaltic wave immediately before drug addition was taken as baseline. After drug administration, the PPT of the last complete peristaltic wave within consecutive 5-min periods (*i.e.*, 5, 10, 15, 20, . . . min) was calculated. Inhibition of peristalsis was reflected by an increase in PPT, and abolition manifested itself in a lack of propulsive motility in spite of an intraluminal pressure of 400 Pa as set by the position of the outlet tubing. Although in this case PPT exceeded 400 Pa, abolition of peristalsis was expressed quantitatively by assigning PPT a value of 400 Pa to obtain numerical results suitable for further statistical evaluation.[20] To obtain the net increase of PPT caused by clonidine or dexmedetomidine, the PPT baseline was subtracted from the respective PPT values recorded in the presence of clonidine or dexmedetomidine.

Statistical Analysis

Quantitative data are presented as medians, interquartile ranges, and 95% confidence intervals. Concentration–response curves were constructed for each experiment with clonidine and dexmedetomidine. The 50% effective concentration values (EC_{50} values) were calculated by the method of Tallarida and Murray.[21]

The results were evaluated statistically with the Kruskal-Wallis H test, if multiple comparisons were made, or with the Mann–Whitney U test or Wilcoxon test for pair differences. A probability value of less than 0.05 was regarded as significant. The software package SPSS for Windows, version 7.0, was used (SPSS Inc., Chicago, IL).

Results

Continuous intraluminal infusion of Tyrode's solution elicited peristaltic contractions (figs. 2 and 3) that stayed constant without any drug addition during the experimental period of 80–100 min. The PPT for eliciting a wave of circular contraction propelling the intraluminal content to the aboral direction ranged between 37 and 68 Pa. The effect of vehicle (Tyrode's solution) and the various antagonists *per se* on PPT were negligible as shown in table 1.

Concentration-dependent Effect of Clonidine and Dexmedetomidine on Peristalsis

Administration of clonidine to the organ bath (10 nM–100 μM) exerted an inhibitory effect on peristaltic motor activity. PPT concentration-dependently increased following exposure to 0.1–30 μM clonidine (figs. 2A and B), and peristaltic activity was completely inhibited by 100 μM clonidine in eight of eight segments tested (fig. 2C). Complete inhibition of peristalsis manifested itself in a lack of propulsive motility in spite of an intraluminal pressure of 400 Pa and occurred 4.3 ± 0.6 min (mean ± SEM) after addition of 100 μM clonidine to the organ bath.

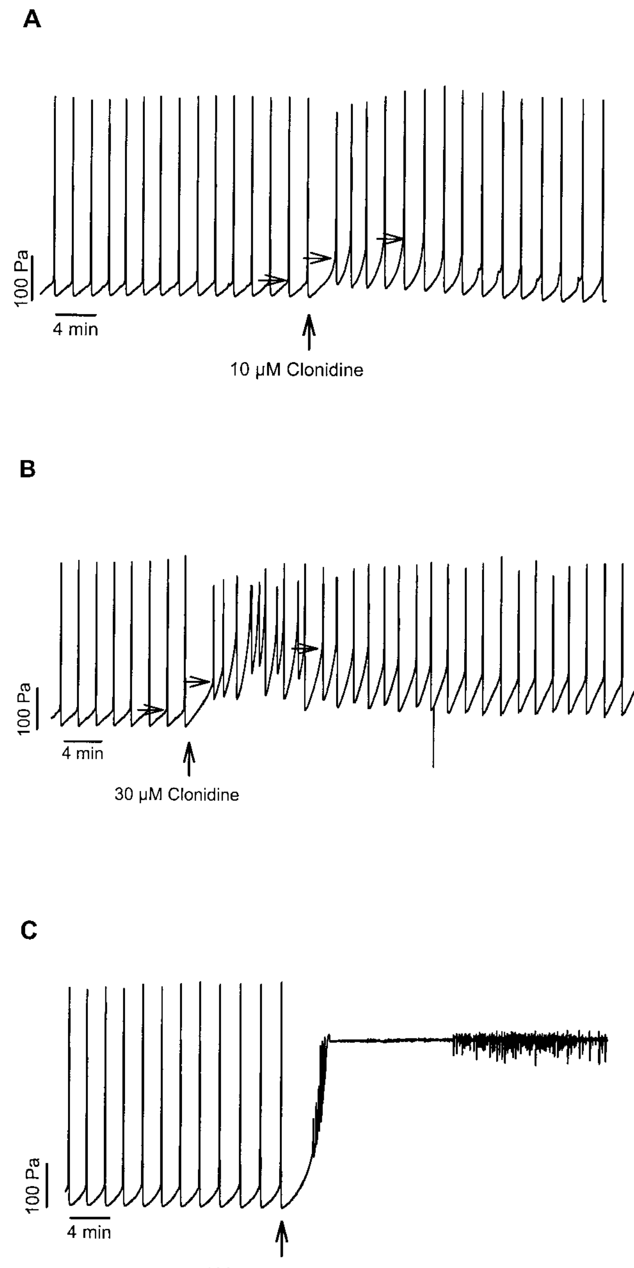

Fig. 2. Original recordings of the effect of clonidine (10–100 μM) on peristalsis in the guinea pig isolated ileum. Peristaltic activity elicited by perfusion of the lumen of the intestinal segment was constant during a control period. When the intraluminal pressure reached the peristaltic pressure threshold (PPT, marked by arrows), a peristaltic wave was triggered, which was measured as a spike-like increase of intraluminal pressure. (*A*) Addition of 10 μM clonidine led to an increase of PPT, which, after approximately 20 min, declined toward predrug level. (*B*) A higher concentration of clonidine (30 μM) further increased PPT, and (*C*) after 100 μM clonidine, peristalsis was completely abolished so that despite an intraluminal pressure of 400 Pa, no peristaltic movements took place.

Dexmedetomidine was more potent in inhibiting peristaltic contractions than clonidine and impaired ileal peristalsis even at a concentration of 3 nM (fig. 3A). The antiperistaltic motor effects of clonidine and dexmedeto-

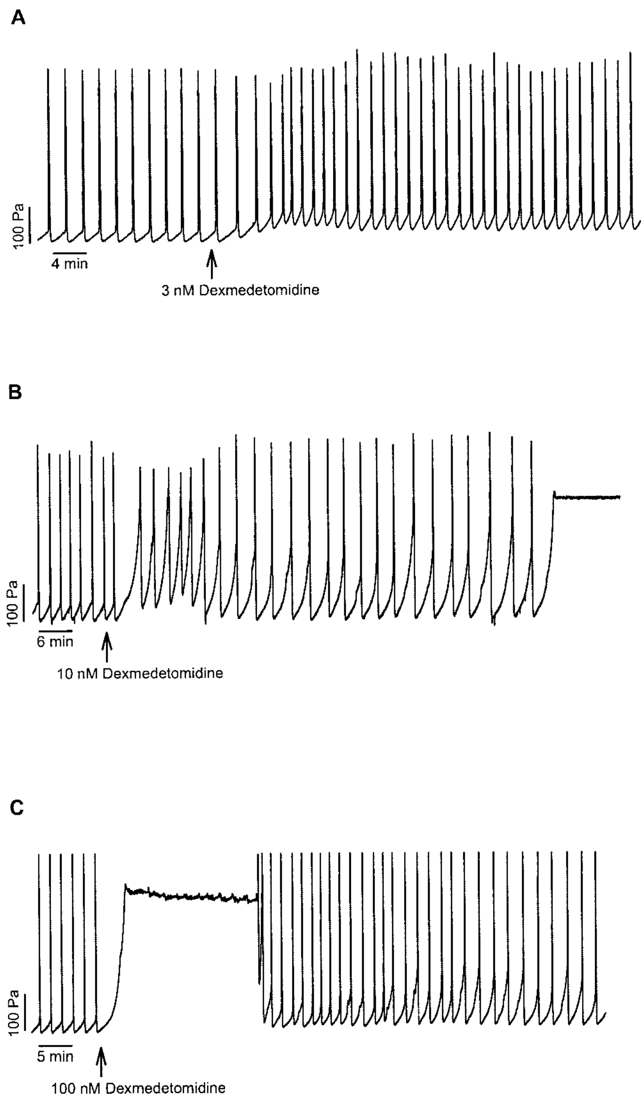

Fig. 3. Effect of dexmedetomidine on ileal peristaltic activity. During the control period after administration of vehicle (Tyrode's solution, not shown in the figure), the intestinal segment displayed regular peristaltic contractions. (*A*) After addition of 3 nM dexmedetomidine to the organ bath, the peristaltic pressure threshold (PPT) increased transiently. (*B*) Dexmedetomidine (10 nM) elicited a marked increase of PPT and after a delay of approximately 60 min peristaltic activity was completely inhibited in this segment. (*C*) A single dose of 100 nM dexmedetomidine immediately abolished peristaltic activity for about 34 min, whereafter peristaltic contractions reoccurred, however, with increased and variable PPTs.

midine were characterized by a rise of PPT, incomplete emptying of the intestinal segments as reflected by an enhanced residual baseline pressure, and an increase in the frequency of peristaltic waves. After the transient increase of PPT due to 3 nM dexmedetomidine, all segments showed regular peristaltic contractions with constant PPTs. At a higher concentration (10 nM), dexmedetomidine caused a more pronounced increase of PPT (fig. 3B), and in four of six segments, peristalsis was completely abolished 19.5 ± 12 min (mean ± SEM) after drug administration. Dexmedetomidine at the highest concentration (100 nM) tested completely inhibited peristalsis (fig. 3C) in four of six segments after 6.0 ± 0.7 min. The complete inhibition of ileal motor activity spontaneously resolved to irregular movements of the intestinal wall in four segments 6.7 ± 0.3 min after the administration of 10 nM dexmedetomidine and in four segments 10.7 ± 3.3 min after 100 nM dexmedetomidine. These irregular movements of the intestinal wall failed to propel the intraluminal content. In the other two segments, regular peristalsis with a PPT similar to that seen during the control period prior to drug administration reoccurred 28.5 min after 10 nM and 23.8 min after 100 nM dexmedetomidine, respectively. The concentration-dependent effect of clonidine (10 nM–100 μM, EC_{50} = 19.6 μM) and dexmedetomidine (0.1–100 nM,

Table 1. Change of PPT (Pa) due to Vehicle and Various Antagonists during a Period of 20 min

	Δ PPT (Pa)	
Vehicle (Tyrode´s solution)	3.5	(0.8 – 6.1)
Yohimbine (1 μM)	0	(−2.9 – 2.2)
Prazosin (1 μM)	0	(−0.7 – 3.2)*
Naloxone (0.5 μM)	−14.5	(−22 – −6)
Apamin (0.5 μM)	1	(−4.5 – 3.8)
L-NAME (300 μM)	18.6	(8.5 – 20.4)
D-NAME (300 μM)	2.6	(0.9 – 5.2)

Data are medians (25^{th}–75^{th} percentiles); n = 8, *n = 14

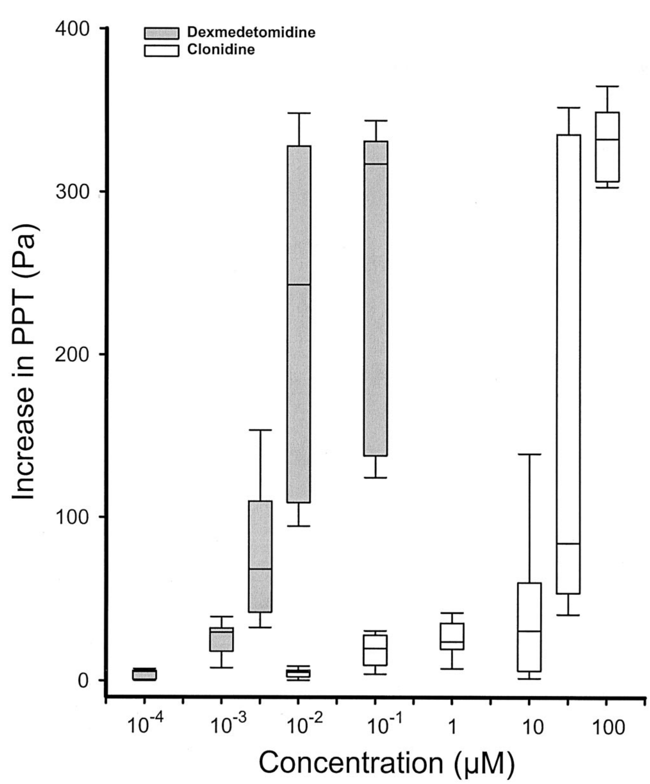

Fig. 4. Concentration–response relation for the increase in PPT (Pa) caused by clonidine (open boxes) and dexmedetomidine (gray boxes). Box plots represent medians, 25th–75th percentiles; whiskers reflect 5th and 95th percentiles (n = 6 for dexmedetomidine, n = 8 for clonidine).

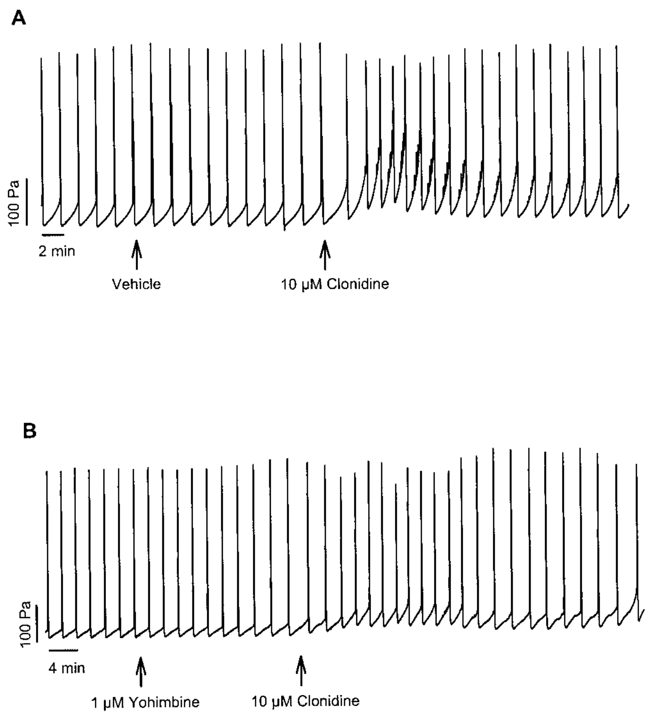

Fig. 5. Clonidine-induced impairment of intestinal peristalsis is mediated through α_2 adrenoceptors. Original recordings of ileal peristaltic contractions after pretreatment with vehicle (Tyrode's solution, *A*) and 1 μM yohimbine (antagonist at α_2 adrenoceptors, *B*) and subsequent application of 10 μM clonidine. Clonidine (10 μM) markedly increased PPT after vehicle pretreatment (*A*), an effect that was attenuated after pretreatment with 1 μM yohimbine (*B*).

EC_{50} = 12.0 nM) on the increase of PPT is summarized in figure 4.

Experiments with Selective Antagonists

To elucidate some of the mechanisms mediating the inhibitory action of clonidine and dexmedetomidine, selective antagonists of putative inhibitory transmitters were added to the organ bath before administration of the respective agonists. The antagonists were tested against submaximally effective concentrations of clonidine (10 μM) and dexmedetomidine (3 nM) in parallel with the respective vehicle solutions. Whereas vehicle (Tyrode's solution) did not impair the inhibitory action of clonidine (10 μM, fig. 5A), the PPT increase due to clonidine was reduced by pretreatment with 1 μM yohimbine ($P < 0.05$, figs. 5B and 6). Pretreatment with 0.5 μM naloxone or 0.5 μM apamin attenuated the clonidine-induced increase in PPT significantly ($P < 0.05$, fig. 6a), whereas 1 μM prazosin failed to exert such a reduction (fig. 6B). Blockade of NO synthase with L-NAME (300 μM) did not affect the clonidine-induced increase in PPT (median, 36.6; 25th–75th percentiles, 25.6–40.4) when compared with the inactive enantiomer D-NAME (300 μM; median, 31.1; 25th–75th percentiles, 29.1–46.8).

The inhibitory action of dexmedetomidine on intestinal peristalsis, visible as an increase of PPT after 3 nM dexmedetomidine, could be reversed by addition of the selective α_2-adrenoceptor antagonist yohimbine (1 μM, fig. 7) or prevented by this drug (fig. 8A). Administration of apamin (0.5 μM, fig. 8A), naloxone (0.5 μM, fig. 8A), and the selective α_1-adrenoceptor antagonist prazosin (1 μM, fig. 8B), however, failed to prevent the increase in PPT due to dexmedetomidine (3 nM).

Discussion

The major findings of the current study are as follows. (1) Clonidine and dexmedetomidine concentration-dependently inhibit peristalsis in the guinea pig small intestine *in vitro*. (2) On a molar basis, dexmedetomidine is much more potent than clonidine. (3) Although both drugs act *via* α_2 adrenoceptors, the mechanism of their antiperistaltic action is in part different. These results were obtained with the *in vitro* technique of Holzer and Maggi,[19] whose major advantage is that intestinal peristalsis can be studied independently of local and systemic blood flow over a long time. In the current organ bath setup, the effects of α_2-adrenoceptor agonists on intestinal peristalsis are not obscured by interference from adrenoceptors on blood vessels, whose activation affects blood supply and, in the case of α_1 adrenoceptors, impairs organ function. The current technique is superior to other *in vitro* preparations, such as the longitudinal muscle nerve preparation of the guinea pig ileum, because it shows effective propulsion of the intraluminal contents in an anal direction and not only changes of muscle force due to a stimulus.

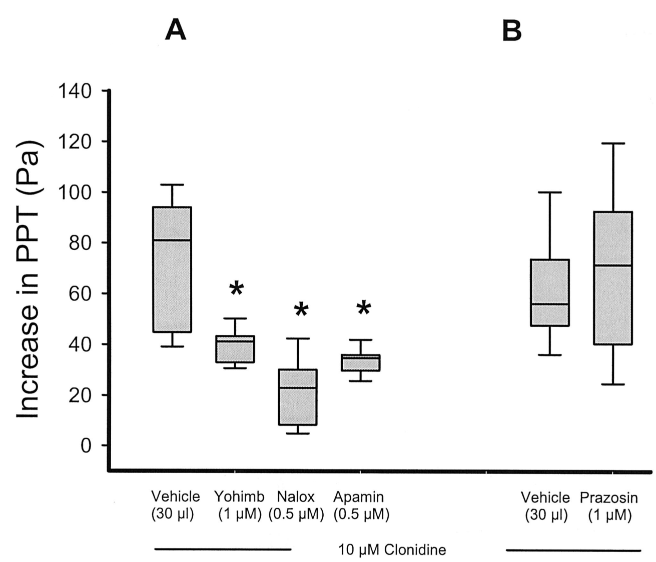

Fig. 6. (*A*) Increase in PPT due to clonidine (10 μM) after pretreatment with vehicle (Tyrode's solution), 1 μM yohimbine (Yohimb), 0.5 μM naloxone (Nalox), and 0.5 μM apamin. (*B*) Increase in PPT due to 10 μM clonidine was not affected by the α_1-adrenoceptor antagonist prazosin (1 μM). Box plots represent medians, 25th–75th percentiles; whiskers reflect 5th and 95^{th} percentiles. **P* < 0.05 as compared with vehicle; (*A*) n = 8, (*B*) n = 14.

By adding drugs into the organ bath, their effects on peristalsis can be studied in a controlled and quantitative fashion that allows the construction of concentration–response curves and the estimation of drug potency and efficacy. It needs to be considered, however, that the drugs administered into the organ bath need to penetrate the serosa and longitudinal muscle before they reach the myenteric plexus where α_2-adrenoceptor agonists are likely to interfere with the neural control of peristalsis.[22] Differences in the diffusion kinetics and metabolic stability of drugs may influence the estimation of their pharmacodynamic characteristics. Further limitations of our approach include the consideration of species differences if the data obtained in the guinea pig small bowel *in vitro* are to be extrapolated to the situation in humans.

Peristalsis is a complex motor pattern of the intestine, which consists of various reflexes coordinated by the intrinsic enteric nervous system.[23] Since it does not depend on inputs from extrinsic neurons, distension-induced peristalsis can be studied in isolated segments of the intestine *in vitro*.[19] In our setup, intraluminal infusion of fluid causes radial distension of the intestinal wall, initially activating an accommodation reflex to relax the circular muscle,[24] which constitutes the preparatory phase of peristalsis. Once the entire segment of intestine is distended to a threshold level, the emptying phase is triggered. The circular muscle at the oral end of the intestine contracts, and then a wave of contraction sweeps anally along the intestinal segment. Ascending excitatory and descending inhibitory reflexes are the basis for these coordinated series of movements involving both the longitudinal and circular muscle layers.[24] While the excitatory motor neurons subserving peristalsis

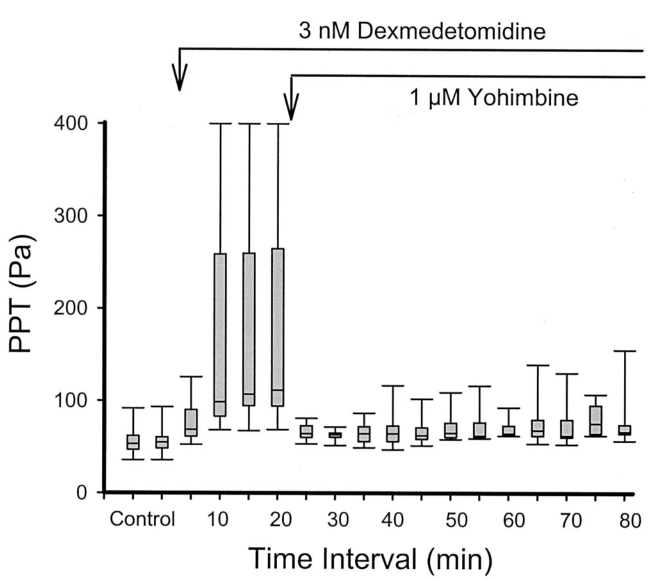

Fig. 7. Yohimbine (1 μM, antagonist at α_2 adrenoceptors) reverses the increase in PPT due to 3 nM dexmedetomidine. Box plots represent medians, 25th–75th percentiles; whiskers reflect 5th and 95th percentiles (n = 8).

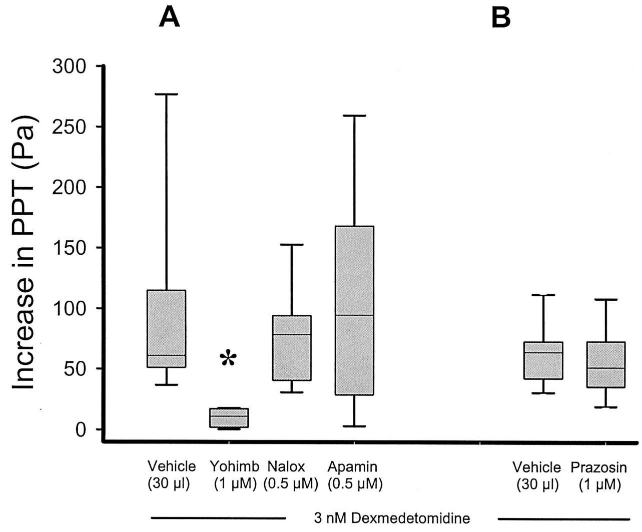

Fig. 8. (*A*) Increase in PPT due to 3 nM dexmedetomidine after pretreatment with vehicle (Tyrode's solution, 30 μl), 1 μM yohimbine (Yohimb), 0.5 μM apamin, and 0.5 μM naloxone (Nalox). The effect of dexmedetomidine was abolished by the α_2-adrenoceptor antagonist yohimbine but remained uninfluenced by apamin and naloxone. (*B*) Increase in PPT due to 3 nM dexmedetomidine was not affected by the α_1-adrenoceptor antagonist prazosin (1 μM). Box plots represent medians, 25th–75th percentiles; whiskers reflect 5th and 95th percentiles. **P* < 0.05 as compared with vehicle; (*A*) n = 8, (*B*) n = 22.

are cholinergic, the inhibitory motor neurons are purinergic and nitrergic as they utilize adenosine triphosphate (ATP) and NO, respectively, as their transmitters.[19,20,22–28]

The antiperistaltic effects of clonidine and dexmedetomidine seen in the current study *in vitro* are consistent with clinical observations,[16–18] experimental studies in humans,[14,15] and suggestions derived from animal experiments.[11–13] The clinical observations have been published as case reports and letters concerning acute colonic pseudoobstruction (Oligivie syndrome) in patients receiving a high dose of clonidine intravenous for treatment of delirium tremens[18] or hypertension.[16,17] Importantly, the inhibitory action of α_2-adrenoceptor agonists on gastrointestinal motility is not restricted to the large bowel, but also seen in other gut regions, such as the stomach and small intestine. By use of the lactulose-breath hydrogen test in healthy volunteers[15,29] and patients,[30] it has been shown that clonidine increases the transit time through the small intestine. However, the lactulose-breath hydrogen test has some limitations[31] since the increase of the mouth-to-cecum transit time might also be due to a delay of gastric emptying. Experiments in the dog have confirmed that α_2-adrenoceptor agonists augment phasic contractions of the pylorus and inhibit antroduodenal motility,[32] but overall, the results concerning the delay of gastric emptying are inconclusive in human and animal studies. In rodents, α_2-adrenoceptor agonists injected subcutaneously did not delay gastric emptying of liquids,[33] whereas in other studies, the agonist had an inhibitory effect.[11] In mice, an overall inhibitory effect of clonidine on gastrointestinal transit has been shown with the intragastric charcoal meal test,[12,13] and a delay of intestinal transit was also noted in rats.[33] In addition to species differences, it has to be considered that gastric emptying and intestinal transport of solid food and liquids are regulated by different mechanisms.[34,35]

Attenuation of intestinal motility by α_2-adrenoceptor agonists *in vivo* is thought to be due to interference with enteric neurons that control muscle activity and fluid secretion. An increase of net transport of fluid from the mucosal to the serosal side[36] and a reduction of motility have led to consider clonidine as an antidiarrheal drug.[37] The current study has shown that another α_2-adrenoceptor agonist, dexmedetomidine, inhibits intestinal peristalsis much more potently than clonidine. A strong inhibition of gastrointestinal transit due to dexmedetomidine was likewise observed in the rat.[11] The 1,000-fold higher potency of dexmedetomidine, compared with clonidine, observed in the current study cannot be accounted for by differences in the affinity of dexmedetomidine and clonidine to α_2 adrenoceptors because the affinity of dexmedetomidine exceeds that of clonidine by a factor of only 3.[10] Since it is unlikely that pharmacokinetic differences account for such a huge potency difference, it is proposed that the mechanisms of the antiperistaltic actions of dexmedetomidine and clonidine differ considerably.

While dexmedetomidine is a highly selective α_2-adrenoceptor agonist, clonidine can act at other adrenoceptors and at imidazoline receptors,[38] although the inhibitory effect of clonidine on enteric neurons does not involve imidazoline receptors.[39] A participation of imidazoline receptors was also ruled out by the finding that the peristaltic motor effects of both clonidine and dexmedetomidine were inhibited by the α_2-adrenoceptor antagonist yohimbine, which lacks activity at imidazoline receptors.[38] In explaining the potency difference between dexmedetomidine and clonidine, it needs to be speculated that, unlike dexmedetomidine, clonidine activates not only α_2 adrenoceptors, but also triggers other mechanisms that counteract the α_2 adrenoceptor–mediated inhibition of peristalsis. This inference is supported by the further pharmacological analysis of the antiperistaltic motor effects of dexmedetomidine and clonidine. The observation that the onset of action of dexmedetomidine was somewhat slower than that of clonidine may also be related to the huge potency difference between the two α_2-adrenoceptor agonists. Given that the effective concentrations of dexmedetomidine were 1,000-fold lower than those of clonidine, it needs to be considered that the concentration gradient driving the diffusion of dexmedetomidine into the intestinal wall was considerably lower than that for clonidine.

The concentrations of clonidine and dexmedetomidine with an inhibitory action in intestinal peristalsis *in vitro* are in the range of those being used clinically or effective in animal models. The lower range of clonidine concentrations (0.1–1 μM) found to impair peristalsis in this study is fairly equivalent to the clinically effective range in the cerebrospinal fluid,[40] whereas concentrations of 10–100 μM are undoubtedly above those reached under clinical conditions in humans. In the rat, the 50% effective plasma concentration of dexmedetomidine required for loss of the whisker reflex is 5.4 nM and for loss of the cornea reflex is 132 nM.[41] However, caution must be exercised in the extrapolation of *in vitro* results to *in vivo* conditions and across species.

It is obvious that in the present study, clonidine and dexmedetomidine inhibit peristalsis through a peripheral mechanism located in the gut wall. Whether central mechanisms contribute to the inhibitory action of α_2-adrenoceptor agonists under clinical conditions is speculative. Centrally mediated inhibition of small intestinal motility has been suggested from experiments in rats and mice in which inhibition of propulsion is most potent when clonidine is given intracerebroventricularly.[42,43] The pharmacological mechanisms behind the peripheral antiperistaltic effect of clonidine and dexmedetomidine were analyzed with a protocol in which transmitter antagonists were tested in parallel with vehicle. Specifically, we have addressed the possibility that clonidine and dexmedetomidine inhibit peristalsis by activating inhibitory α_1 adrenoceptors on the smooth muscle by activating inhibitory α_2 adrenoceptors on excitatory cholinergic pathways in the enteric nervous system[22] or by activating inhibitory neural pathways such as opioidergic, purinergic, and nitrergic neurons.[26] Inhibitory motor transmission in the guinea pig intestine can be blocked by a combination of apamin, which blocks small conductance Ca^{2+}-dependent potassium channels operated by ATP,[25,26,44,45] and an inhibitor of NO synthase.[27,28] Therefore, effective and selective concentrations of the α_1-adrenoceptor antagonist prazosin (1 μM[46]), the α_2-adrenoceptor antagonist yohimbine (1 μM[46]), the opioid receptor antagonist naloxone (0.5 μM[20]), the NO synthase inhibitor L-NAME and its inactive enantiomer D-NAME (each at 300 μM[47]), and apamin (0.5 μM[25]) were employed to analyze the receptor and mediator mechanisms behind the antiperistaltic motor responses to clonidine and dexmedetomidine.

The inhibitory effect of clonidine and dexmedetomidine on intestinal peristalsis was prevented or reversed by the selective α_2-adrenoceptor antagonist yohimbine, which confirms that both drugs act *via* α_2 adrenoceptors and that their antiperistaltic effects are due to α_2 adrenoceptor–mediated interruption of excitatory cholinergic pathways in the enteric nervous system.[22] In contrast, prazosin failed to significantly alter the motor responses to dexmedetomidine and clonidine, which rules out a major implication of α_1 adrenoceptors. It is generally accepted that in the gut, postsynaptic α adrenoceptors belong to the α_1 class, while those on the terminals and possibly cell bodies of enteric cholinergic neurons are of the α_2 subtype.[22,48] Our findings indicate, therefore, that clonidine and dexmedetomidine inhibit peristalsis by an action on enteric neurons, which is in keeping with the ability of α_2-adrenoceptor agonists to inhibit the release of acetylcholine from the guinea pig ileum.[49]

Unlike the antiperistaltic effect of dexmedetomidine, which was suppressed by yohimbine only, the rise of PPT caused by clonidine was also attenuated by naloxone and apamin. First, this finding is in line with the enhanced α_2-adrenoceptor selectivity of dexmedetomidine as compared with that of clonidine.[10] Second, this finding indicates that clonidine differs from dexmedetomidine inasmuch as it impairs peristalsis not only through α_2 adrenoceptor–mediated inhibition of enteric cholinergic transmission, but also through stimulation of inhibitory enteric neural pathways, such as opioidergic enteric neurons and inhibitory motor neurons that release a transmitter signaling *via* apamin-sensitive potassium channels. The molecular mechanism of this particular action of clonidine, as opposed to that of dexmedetomidine, awaits to be elucidated. In contrast, nitrergic transmission does not seem to be involved in the inhibitory motor action of clonidine because blockade of NO synthase with L-NAME was without effect. These data illustrate two important conclusions: (1) Intestinal peristalsis can be suppressed by α_2-adrenoceptor agonists through a peripheral site of action on enteric neurons in the gut, and (2) the enteric neurons targeted by clonidine and dexmedetomidine differ to a considerable extent.

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