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Effects of antagonism of NMDA receptors on transient lower esophageal sphincter relaxations in the dog

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Abstract

Transient lower esophageal sphincter relaxation is the major cause of gastroesophageal reflux. Mechanisms underlying transient lower esophageal sphincter relaxation are poorly understood although gastric mechanosensitive vagal afferent pathways play a central role. Glutamate is a key transmitter of vagal afferents acting partly on NMDA receptors. The aim of this work was to study the effects on transient lower esophageal sphincter relaxation in awake dogs ($n = 5$) of the competitive NMDA receptor antagonist *cis*-4-phosphonomethyl-2-piperidine carboxylic acid (CGS 19755; 0.3 and 3 mg/kg i.v., the high dose was given at two separate occasions to each dog). Transient lower esophageal sphincter relaxations were evoked by intragastric infusion of a liquid meal followed by air insufflation and were scored during a 45-min period. Neither dose produced any significant effect on the group average number of transient lower esophageal sphincter relaxations. Synchronous contractions of the esophagus were commonly seen during transient lower esophageal sphincter relaxation and CGS 19755 at both doses greatly reduced their occurrence. The findings indicate that NMDA receptor antagonism selectively inhibits the esophageal component of transient lower esophageal sphincter relaxation although the rate of transient lower esophageal sphincter relaxations is not consistently affected.

Keywords: Vagus; Glutamate; Dorsal vagal complex; Motility; Gastro-esophageal reflux

1. Introduction

Gastro-esophageal reflux was earlier considered to occur primarily as a result of a chronically low or absent lower esophageal sphincter pressure. More recent studies using methods better suited for continuous measurement of lower esophageal sphincter pressure suggest, however, that transient, complete relaxations of the sphincter are considerably more important as a mechanism for reflux (Dent et al., 1980; Holloway et al., 1991; Mittal et al., 1995b). These episodic relaxations, known as transient lower esophageal sphincter relaxations, are increased in number postprandially (Dent et al., 1980; Holloway et al., 1991) and are triggered chiefly by gastric distension (Franzi et al., 1990; Holloway et al., 1985). The physiological relevance of transient lower esophageal sphincter relaxation is probably venting of gas from the stomach but the mechanism seems to be imperfect as fluid reflux occurs occasionally, which in some individuals results in reflux disease.

Activation of gastric mechanoreceptors with sensory endings primarily in the subcardiac region (Franzi et al., 1990) is the most powerful stimulus for increased occurrence of transient lower esophageal sphincter relaxation that is mediated via a vago-vagal pathway (Martin et al., 1986). Triggering of transient lower esophageal sphincter relaxation is influenced by a number of factors such as hormonal status (Boulant et al., 1994), posture (Little et al., 1989) and level of consciousness (Cox et al., 1988). The efferent signal is not only carried by vagal but also by phrenic efferents supplying the crural diaphragm (Mittal et al., 1995b). Simultaneous relaxation of the crural diaphragm and lower esophageal sphincter is a prerequisite for ablation of the pressure difference across the gastroesophageal junction. Consequently, the most rational way to inhibit transient lower esophageal sphincter relaxations may be to block afferent activity from gastric mechanoreceptors. There is a paucity of data on pharmacological interventions aimed at inhibiting transient lower esophageal sphincter relaxations. Blockade of CCK_A (Boulant et al., 1994) and muscarinic receptors (Mittal et al., 1995a), as well as stimulation of $GABA_B$ (Blackshaw et al., 1999; Lehmann et al., 1999) and opioid receptors (Penagini and

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Bianchi, 1997) or inhibition of nitric oxide synthase (Boulant et al., 1994) has been shown to reduce transient lower esophageal sphincter relaxation incidence.

There are several putative neurotransmitters present in the vagal afferent fibers. These include neuropeptides such as substance P, calcitonin gene-related peptide and the amino acid glutamate. Glutamate is considered to be the quantitatively most important excitatory transmitter in the central nervous system where it acts on NMDA, kainate, AMPA and metabotropic glutamate receptors. There is substantial evidence suggesting that glutamate serves a transmitter function in vagal afferents. Electrical stimulation of the vagus evokes release of glutamate in the nucleus of the tractus solitarius (Allchin et al., 1994). A subpopulation of cell bodies of the nodose ganglion are immunoreactive for glutamate (Sykes et al., 1997), and there seem to be glutamate receptors both on peripheral (Cincotta et al., 1989) and central portions (Lewis et al., 1988) of the vagal afferents. Pharmacological evidence suggests that both NMDA and non-NMDA (Andresen and Yang, 1990; Lu and Bieger, 1998; Travagli et al., 1990) receptors are involved in vagal afferent transmission. NMDA receptors are involved in central integration of swallowing (Lu and Bieger, 1998; Hashim et al., 1989) and as transient lower esophageal sphincter relaxation may share some of the central pathways with swallowing (Mittal et al., 1995b), it is possible that inhibition of NMDA receptors may influence transient lower esophageal sphincter relaxation.

The present work aimed at determining the effects of a well characterized (Lehmann et al., 1988) competitive NMDA receptor antagonist, *cis*-4-phosphonomethyl-2-piperidine carboxylic acid (CGS 19755), on transient lower esophageal sphincter relaxation in Labrador retrievers. CGS 19755 has neither any significant effect on other glutamate receptors nor does it have any affinity for several other transmitter receptors (Lehmann et al., 1988).

2. Materials and methods

Cervical esophagostomies were made in five Labrador retrievers (four males; age 1.5–2 years). The method used allowed rapid recovery and all dogs were able to eat wetted food the day after surgery. There was no leakage of food but some leakage of water through the esophagostomy. The dogs weighed between 20 and 30 kg during the experimental period. Because the dogs showed an interindividual variability in duplicate experiments after administration of CGS 19755, the individual results are presented with reference to dogs 1–5 where dog 5 was a female and the rest were males. Dogs 2 and 4, and 3 and 5, respectively, were littermates whereas dog 1 was from a separate litter. After recovery from surgery, the dogs were accustomed to rest in a Pavlov stand. At the time of the experiment, the dogs had been fasting for approximately 16–18 h, but water was freely supplied. All procedures were approved by the Ethical Committee for Animal Experiments of the Göteborg region.

Transient lower esophageal sphincter relaxations and esophageal motility were measured using Dentsleeve manometry as described by Stakeberg and Lehmann (1999) and by Lehmann et al. (1999) with the following modification: The esophageal side-holes were perfused at 0.1 ml/min and the lower esophageal sphincter and intragastric channels were perfused at 0.45 ml/min.

Following intubation, a baseline recording was made for at least 10 min. Placebo or drug (0.5 ml/kg) was then injected during 2 min in a foreleg vein. Any behavioural reactions were noted after drug administration. Ten minutes later, an acidified nutrient (30 ml/kg, room temperature) was infused using a peristaltic pump at 100 ml/min through a central channel of the multilumen assembly. The nutrient consisted of 10% peptone (w/v; Difco Labs., Detroit, MI), 5% Intralipid (v/v; Pharmacia, Stockholm, Sweden) and 5% D-glucose (w/v; Kebo Lab., Spånga, Sweden) and pH was titrated with HCl to 3.0. The nutrient was usually made the day before the experiment and kept refrigerated overnight and in no instance was it more than 2 days old. Directly after administration of nutrient, air was insufflated into the stomach at a rate of 40 ml/min. The total time of nutrient infusion and air insufflation was 45 min. The peristaltic pump was calibrated at 40 and 100 ml/min before each experiment.

The definition of transient lower esophageal sphincter relaxation was identical to that used previously (Stakeberg and Lehmann, 1999; Lehmann et al., 1999) with the following modification: The duration of complete sphincter relaxation was set at > 0.5 s.

Although not used in the inclusion criteria, identification of transient lower esophageal sphincter relaxation was facilitated by certain other events. Transient lower esophageal sphincter relaxations were mostly accompanied by simultaneous contraction of the esophagus in control experiments (see Results). Further, reflux of acidic gastric contents often occurred during transient lower esophageal sphincter relaxation. In addition, a belch or passing of air through the esophagostomy was frequently heard, and common cavities (equalisation of gastric and esophageal pressures) could occasionally be seen. Esophageal motility was analysed using the criteria described by Stakeberg and Lehmann (1999) and by Lehmann et al. (1999).

CGS 19755 was synthesized by AstraZeneca R&D Södertälje (Södertälje, Sweden) or purchased from Sigma-RBI (Natick, MA, USA). CGS 19755 was given at 0.3 mg/kg (4.5 μmol/kg) once to each dog, and at 3.0 mg/kg (45 μmol/kg) to all dogs at two separate occasions, and drug from both sources were given to each dog. The doses selected were based on findings in rodents (Lehmann et al., 1988) taking into account that dogs usually require lower doses than rodents. A higher dose (8

mg/kg) was also tested but not pursued any further since it produced behavioural side effects. The average of the results from five previous experiments per dog in which 0.9% saline was used as placebo served as control so the entire experimental series consisted of 40 separate manometric recordings. The time from the first to the last placebo experiment before drug test was 9.5 ± 3.5 months (mean ± S.E.M.). We have observed that there is no time-dependent change in the control number of transient lower esophageal sphincter relaxation or any related motility variable in our dog colony (longest observation period: 6 years).

The results are presented as individual data or as mean ± S.E.M. Student's paired *t*-test was used for the statistical analysis unless otherwise stated.

3. Results

Spontaneous transient lower esophageal sphincter relaxations before gastric distension with fluid and air were rare. The average number of transient lower esophageal sphincter relaxations for the period of measurement (45 min) of five control experiments per dog was as follows: dog 1, 5.2 ± 0.4; dog 2, 8.6 ± 0.4; dog 3, 2.4 ± 0.3; dog 4, 9.2 ± 0.9; dog 5, 9.3 ± 0.4. A large number of experiments performed in our laboratory have verified that interindividual variability with respect to frequency of transient lower esophageal sphincter relaxation is fairly pronounced in contrast to intraindividual variability.

Basal lower esophageal sphincter pressure fluctuated greatly and could sometimes be absent in the preprandial

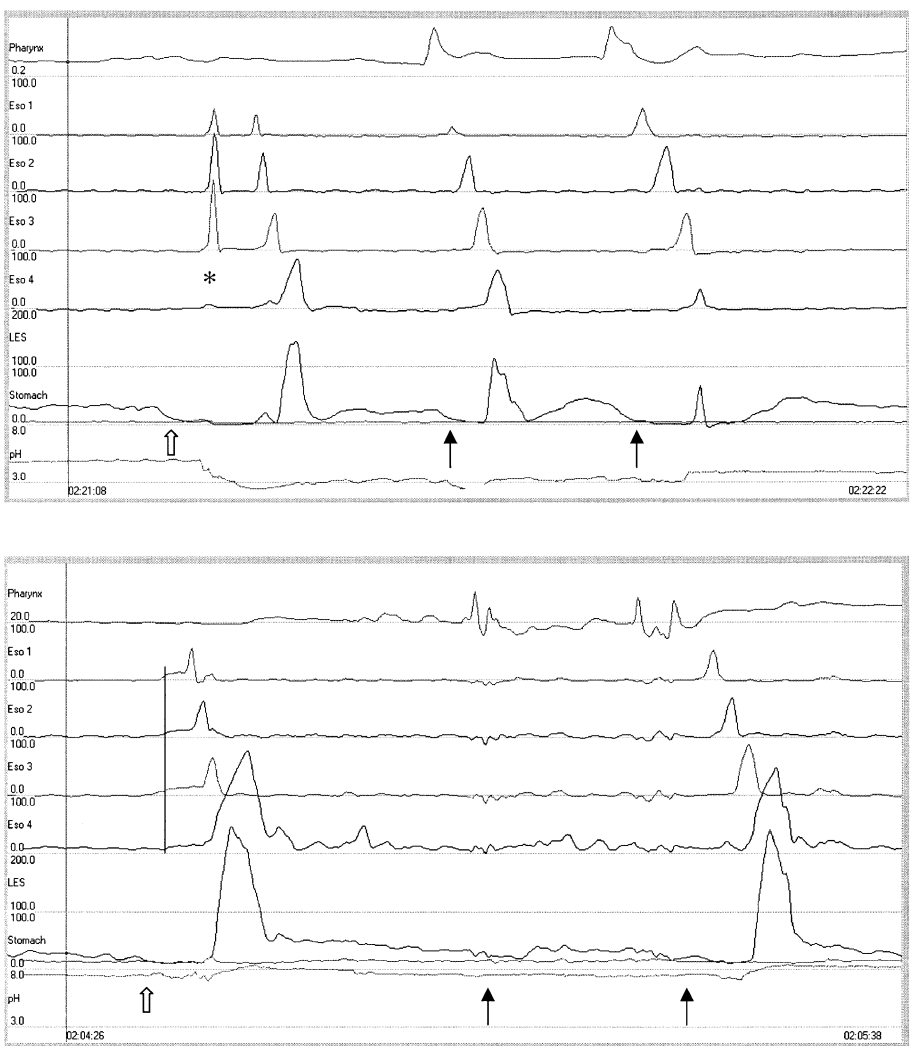

Fig. 1. Original tracing of transient lower esophageal sphincter relaxation in a control experiment (upper panel) and after 3 mg/kg CGS 19755 in dog 5 (lower panel). Pressures on the *Y*-axis are given in mm Hg; "Eso 1–4" correspond to the esophageal ports (scale 0–100 mm Hg). For the sake of clarity, lower esophageal sphincter and stomach pressures were superimposed (scale 0–200 mm Hg). Transient lower esophageal sphincter relaxations are marked with unfilled arrows and swallows with arrows. In the upper tracing, the transient lower esophageal sphincter relaxation is accompanied by contraction at the upper three esophageal sites and acid reflux. A secondary peristaltic wave terminates the transient lower esophageal sphincter relaxation. Both swallows are followed by successful peristalsis. In the lower tracing, there is no esophageal contraction during the transient lower esophageal sphincter relaxation. In this case, there is no acid reflux but gas reflux as reflected by the esophageal common cavity (marked by vertical line). The transient lower esophageal sphincter relaxation ends with secondary peristalsis. The first swallow is not followed by esophageal peristalsis while the second is.

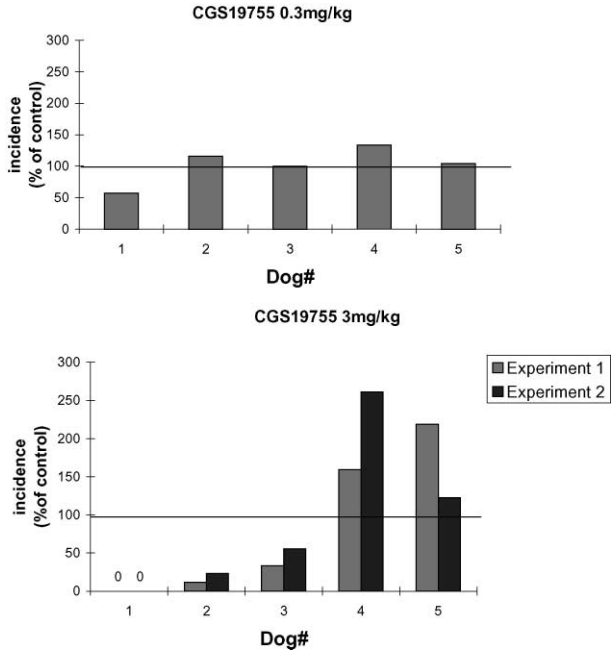

Fig. 2. Effects of CGS 19755 on transient lower esophageal sphincter relaxation in the dog. The upper panel shows the results obtained with 0.3 mg/kg CGS 19755 (one experiment per dog) and the lower panel shows the effects of 3 mg/kg CGS 19755 (two experiments per dog). Average control level of transient lower esophageal sphincter relaxations for every 45 min was 6.8 ± 1.4. The probability of obtaining a subject-related reproducible inhibition or stimulation in terms of number of transient lower esophageal sphincter relaxations was statistically significant ($P = 0.03$).

state. In those cases, it always increased rapidly upon infusion of nutrient. After nutrient infusion, lower esophageal sphincter pressure was generally higher but could still be very variable (Stakeberg and Lehmann, 1999). Reflux of acidic contents was almost always caused by transient lower esophageal sphincter relaxation (Stakeberg and Lehmann, 1999).

A rapid increase in pressure in the three proximal esophageal channels occurred after the onset of transient lower esophageal sphincter relaxation in $97 \pm 1\%$ of the cases (Fig. 1). This increase reflected contraction of the esophageal body and usually appeared when the LES was fully relaxed. In some instances, the contraction was seen shortly after complete relaxation but it never occurred before. In addition, primary and/or secondary peristalsis was almost always observed during and after the transient lower esophageal sphincter relaxation (Stakeberg and Lehmann, 1999; Fig. 1).

3.1. Effects of CGS 19755 on transient lower esophageal sphincter relaxation

CGS 19755 at 0.3 and 3.0 mg/kg did not cause any visible side effects in any of the dogs. After 3.0 mg/kg CGS 19755, the number of transient lower esophageal sphincter relaxations was $91 \pm 30\%$ of control. However, in three dogs (dogs 1–3, all males), there was a reduction of transient lower esophageal sphincter relaxations as compared with their respective control value (Fig. 2). In the two other dogs (one female and one male), there was an increase in the number of transient lower esophageal sphincter relaxations (Fig. 2). These findings were replicated in a second experiment using 3 mg/kg of CGS 19755 (Fig. 2). The probability that the change in transient lower esophageal sphincter relaxation incidence would go in the same direction in two tests in five dogs by coincidence is $1/2^5$, i.e. $P = 0.031$. Since each dog was given CGS 19755 from both suppliers, the source of the drug cannot account for this finding. The lower dose of CGS 19755 did not produce any significant changes in the incidence of transient lower esophageal sphincter relaxation ($102 \pm 13\%$ of control; Fig. 2).

The latency from start of nutrient infusion to the first transient lower esophageal sphincter relaxation was 3.4 ± 1.2 min in control experiments (Table 1). Although not statistically significant ($P = 0.074$), this was increased to $430 \pm 175\%$ of control after CGS 19755 at 3 mg/kg. There was no simple relationship between latency and number of transient lower esophageal sphincter relaxation although the three dogs that had a reduced number of transient lower esophageal sphincter relaxations also had longer latencies in 5/6 experiments. The two dogs that were stimulated by CGS 19755 showed latencies that were close to control values. At 0.3 mg/kg, CGS 19755 did not seem to affect the latency ($172 \pm 99\%$ of control; Table 1).

Table 1
Effect of CGS 19755 on latency to first transient lower esophageal sphincter relaxation and duration of transient lower esophageal sphincter relaxation

	Vehicle	CGS 19755, 0.3 mg/kg (percent of control)	CGS19755, 3 mg/kg (percent of control)
Latency to first transient lower esophageal sphincter relaxation (min)	3.4 ± 1.2	172 ± 99	430 ± 175 ($P = 0.074$)
Duration of transient lower esophageal sphincter relaxation (s)	8.7 ± 0.7	74 ± 4[a]	84 ± 8

Latency was calculated as the time between start of infusion of nutrient to the first transient lower esophageal sphincter relaxation. The results represent mean ± S.E.M., $n = 5$. The controls were based on five experiments per dog and the high dose of CGS 19755 was given at two different occasions to each dog.

[a] $P < 0.01$

Table 2
Effect of CGS 19755 on esophageal motility

	Vehicle	CGS 19755, 0.3 mg/kg (percent of control)	CGS 19755, 3 mg/kg (percent of control)
Simultaneous contraction (percent of all transient lower esophageal sphincter relaxations)	97 ± 1%	1.4 ± 0.9[a]	0 ± 0[a]a
Peristaltic velocity (cm/s)	3.3 ± 0.1	91 ± 1[b]	112 ± 10
Amplitude[c] (mm Hg)	64.9 ± 3.0	104 ± 8	107 ± 10
Duration of contraction[c] (s)	1.7 ± 0.1	92 ± 6	106 ± 7

Peristaltic parameters were calculated for swallows only.
[a] $P < 0.001$.
[b] $P < 0.01$.
[c] Eight centimeters proximal to the midpoint of the sleeve.

The duration of transient lower esophageal sphincter relaxation was significantly reduced only after the lower dose of the NMDA receptor antagonist (Table 1).

3.2. Effects of CGS 19755 on esophageal motility

With the exception of a minor but statistically significant reduction in peristaltic velocity after 0.3 mg/kg of CGS 19755, none of the peristaltic parameters was significantly changed (Table 2). However, CGS 19755 very markedly reduced the occurrence of simultaneous contractions of the esophageal body (Fig. 1; Table 2). After the high dose, none of the transient lower esophageal sphincter relaxations was accompanied by simultaneous contractions. Only 1.4 ± 0.9% of the transient lower esophageal sphincter relaxations were accompanied by esophageal contraction after CGS 19755 at 0.3 mg/kg.

4. Discussion

The motor events during a transient lower esophageal sphincter relaxation are complex and consist of changes not only in lower esophageal sphincter but also in esophageal body, gastric and crural diaphragm motility (Mittal et al., 1995b). The observation that simultaneous contractions of the esophageal body during a transient lower esophageal sphincter relaxation were abolished by CGS 19755 demonstrates for the first time that motor events associated with transient lower esophageal sphincter relaxations can be pharmacologically separated. Other compounds affecting transient lower esophageal sphincter relaxations such as $GABA_B$ receptor agonists (Blackshaw et al., 1999; Lehmann et al., 1999) and nitric oxide synthase inhibitors (Boulant et al., 1994) reduce the incidence without altering the morphology of transient lower esophageal sphincter relaxations. This indicates that the inhibition of simultaneous esophageal contraction by CGS 19755 takes place downstream of the first synapse in the nucleus of the solitary tract in the reflex arc triggering transient lower esophageal sphincter relaxation. The most likely site of action for this effect is esophageal motor neurons of the compact formation of the nucleus ambiguus, which express mRNA for the NMDA receptor (Broussard et al., 1994). Although the present data does not permit conclusions on the site of action of CGS 19755 with regard to inhibition of simultaneous esophageal body contraction, it may be speculated that massive and synchronous excitation of esophageal motor neurons driven by a central pattern generator during transient lower esophageal sphincter relaxation (Mittal et al., 1995b) is mediated by NMDA receptors. An alternative hypothesis is that different groups of vagal afferents control triggering of transient lower esophageal sphincter relaxation and simultaneous contraction of the esophagus. This hypothesis seems less likely since the contraction never occurs in isolation and since other motility events during transient lower esophageal sphincter relaxation appear to be controlled by a central pattern generator (Mittal et al., 1995a,b). There were minor effects on transient lower esophageal sphincter relaxation duration and esophageal peristalsis after CGS 19755. However, since they did not seem to be dose-dependent, their significance is uncertain.

The present study shows that there is no simple relationship between antagonism of NMDA receptors and occurrence of transient lower esophageal sphincter relaxation. Rather, the results indicate that there may be dual effects of the NMDA receptor antagonist CGS 19755 on transient lower esophageal sphincter relaxation, depending on the individual studied, but this suggestion needs further experimental support. The absence of any clear effects of transient lower esophageal sphincter relaxation incidence does not mean that glutamate is not a transmitter in the gastric vagal afferents initiating transient lower esophageal sphincter relaxation. It is possible that a higher dose of an NMDA present antagonist is required to inhibit transient lower esophageal sphincter relaxations but results from such an experiment would be inconclusive because of accompanying behavioural effects. Another possibility is that non-NMDA receptors of either the ionotropic (Willis et al., 1996) or metabotropic (Glaum and Miller, 1992) subtypes may mediate effects of glutamate.

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