The acid pocket, hiatal hernia and TLESRs: essential players in the pathogenesis of gastro-esophageal reflux disease

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The role of $\text{GABA}_A$ receptors in the control of transient lower esophageal sphincter relaxations in the dog

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Abstract

Background and purpose: Transient lower esophageal sphincter relaxations (TLESRs) are triggered by activation of mechanosensitive gastric vagal afferents and are the major cause of gastroesophageal reflux and therefore an important target for therapeutic intervention in gastroesophageal reflux disease (GERD). Activation of the metabotropic GABA\textsubscript{B} receptor has shown to inhibit TLESRs. The aim of the present study was to assess the role of the ionotropic GABA\textsubscript{A} receptor in the regulation of TLESR.

Experimental approach: TLESRs were quantified using Dentsleeve manometry in dogs, and GABA\textsubscript{A} agonists were dosed i.v. prior to gastric distension. Immunohistochemistry and RT-PCR were used to localize GABA\textsubscript{A} receptors in the dog nodose ganglion, the source of vagal afferents which initiate TLESRs.

Key results: The prototypical GABA\textsubscript{A} agonist muscimol produced a dose-dependent inhibition of TLESRs ranging from 19 to 56%. The two other GABA\textsubscript{A} agonists evaluated, isoguvacine and 4,5,6,7-tetrahydroisoaxazolo-[5,4-c]pyridin-3-ol (THIP), as well as the GABA\textsubscript{A} positive allosteric modulator diazepam, had no major effects on TLESRs. Evaluation of higher doses was limited by emesis (THIP and isoguvacine) or restlessness/sedation (diazepam). Of the predominant GABA\textsubscript{A} subunits (the \(\alpha\), \(\beta\) and \(\gamma\) isoforms), \(\alpha\) and \(\beta\) but not \(\gamma\) were detected in the dog nodose ganglion by RT-PCR, while immunohistochemistry in addition demonstrated nerve fibres expressing the \(\gamma\) subunit.

Conclusions and implications: The present observations demonstrate that GABA\textsubscript{A} receptors exert an inhibitory control of TLESRs. These results warrant further studies using GABA\textsubscript{A} isoform-selective agonists to define the identity of receptors involved.
Introduction

Gastroesophageal reflux disease (GERD) is one of the most common disorders of the GI tract and is characterized by symptoms of heartburn, regurgitation and retrosternal pain.\(^1,2\) Transient lower esophageal sphincter relaxation (TLESR) is the major mechanism underlying gastro-esophageal reflux, both in healthy volunteers and in GERD patients.\(^3\) Pharmacological inhibition of TLESRs is therefore a potential target for the treatment of GERD. TLESRs are triggered by gastric distension, leading to a vagally mediated reflex pathway involving mechanosensitive gastric vagal afferents, integrative brainstem centres and vagal efferents to the lower esophageal sphincter (LES).\(^4\)

\(\gamma\)-Aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the central nervous system, acting through either ionotropic GABA\(_A\) and GABA\(_C\) or metabotropic GABA\(_B\) receptors. The GABA\(_{A/C}\) receptors are ligand gated ion channels, which induce rapid synaptic inhibition upon activation. This contrasts to the GABA\(_B\) receptor, which couples to G-proteins and produce longer lasting inhibitory signals.\(^5\) Inhibitory GABA\(_B\) receptors are present on gastric mechanoreceptors\(^6\) and on vagal afferent terminals in the dorsal medulla and have shown to inhibit transmitter release in vagal nuclei.\(^7\) Previous studies showed that the GABA\(_B\) receptor agonist baclofen significantly reduces the rate of TLESRs in dogs, ferrets and humans.\(^8-11\) Baclofen also reduces the number of reflux episodes and reflux symptoms in GERD patients.\(^12-14\) However, the side effect profile of baclofen makes it less attractive for clinical use. It is unknown if ionotropic GABA\(_A\) receptors play a similar role as GABA\(_B\) receptors.

GABA\(_A\) receptors are also widely expressed in the central and peripheral nervous systems and mediate fast postsynaptic inhibition. The GABA\(_A\) receptor complex is a pentameric assembly of subunits forming a chloride channel and, depending on subunit configuration, benzodiazepine, barbiturate and neuroactive steroid sites.\(^15\) The various subunits of the GABA\(_A\) receptors are \(\alpha 1-\alpha 6\), \(\beta 1-\beta 3\), \(\gamma 1-\gamma 3\) and \(\delta\).\(^16\) Each GABA\(_A\) receptor consists of a combination of various subunits. Co-expression of the \(\alpha\), \(\beta\) and \(\gamma\) subunits is required for the formation of a fully functional GABA\(_A\) receptor.\(^17\) GABA and other directly acting GABA\(_A\) receptor agonists bind specifically to a recognition site located between an \(\alpha\) and a \(\beta\) subunit, whereas the benzodiazepine ligands bind to an allosteric site located between an \(\alpha\) and a \(\gamma\) subunit.\(^18,19\) However, for the formation of the GABA\(_A\) receptor, co-localization of these three types of subunits is not an absolute requirement. It is known that mice lacking the \(\gamma 2\) subunit were able to express functional GABA\(_A\) receptors, but were lacking the benzodiazepine binding site.\(^20\) Other studies on recombinant GABA\(_A\) receptors also showed that the combination of the \(\alpha 1\) and \(\beta 2\) subunits produces functional GABA\(_A\) receptors, but the \(\gamma 2\) subunit is required to express benzodiazepine binding.\(^21,22\) Positive GABA\(_A\) modulators, like benzodiazepines, facilitate GABA mediated Cl\(^-\) flux and have sedative, anxiolytic and anticonvulsant effects.

GABA\(_A\) agonism has been found to excite \(^23\), inhibit \(^24\) or have no effect on vagal afferents, the key initiators of TLESRs. Whether these contradictory results are due to species or methodological differences is unknown, but they warrant studies on the effects...
of peripherally restricted agonists on TLESRs. On the other hand, GABA<sub>A</sub> receptors mediate inhibition in the dorsal vagal complex, the central relay station translating afferent signalling into efferent firing producing TLESRs. It can therefore be speculated that central GABA<sub>A</sub> agonism may modify the peripheral actions of GABA<sub>A</sub> agonists.

These hypotheses were tested by assessing the potential for peripheral agonistic effects by determining GABA<sub>A</sub> receptor subunit expression in the dog nodose ganglion (NG), the origin of vagal afferents. Furthermore, to characterize peripheral and central involvement of GABA<sub>A</sub> receptors in the regulation of TLESRs in the dog, the effects of two centrally acting GABA<sub>A</sub> agonists, muscimol and THIP (4,5,6,7-tetrahydroisoxazolo [5,4-c] pyridine-3-ol), and the non-selective, peripherally acting agonist isoguvacine, as well as the positive GABA<sub>A</sub> modulator diazepam were studied. The selection of a benzodiazepine and THIP was based on the finding that the former selectively enhances the function of synaptic α1β2γ2S GABA<sub>A</sub> receptors while the latter preferentially activates extrasynaptic α4β3δ GABA<sub>A</sub> receptors.¹⁹

Materials and methods

Dog experiments

Animals

Adult male and female Labrador retrievers were used in the experiments. Cervical esophagostomies were made and after recovery from surgery the dogs were accustomed to rest in a Pavlov stand. Before each experiment, the dogs were fasted overnight with free access to water. A washout of at least three days was allowed between experiments. All procedures were approved by the Ethical Committee for Animal Experiments of the Göteborg region.

Protocol

The dogs were intubated with a water-perfused Dentsleeve multilumen catheter to record gastric, lower esophageal sphincter (LES) and esophageal pressures. An antimony pH electrode was placed 3 cm above the lower esophageal sphincter to measure acid reflux episodes and a water-perfused catheter was placed in the hypopharynx to measure swallows.

TLESRs were stimulated by infusion into the stomach through the central lumen of the assembly of a acidified liquid nutrient (30 ml kg⁻¹) followed by air insufflation (100 ml min⁻¹) to maintain gastric pressure between 9-11 mmHg.

The number of TLESRs was measured during a 45 minute period starting from the infusion of the liquid. TLESRs were defined by a rapid decrease of LES pressure (> 1 mmHg s⁻¹) to a pressure < 2 mmHg above gastric pressure and a duration of > 1s, without any pharyngeal signal < 2s before onset.⁹

Muscimol, isoguvacine and diazepam were administered as i.v. boluses (muscimol and isoguvacine 0.5 ml kg⁻¹, diazepam 1 mg kg⁻¹ containing 5 mg ml⁻¹) 10 min before start of the experiment. THIP was administered according to an infusion protocol of 1.0 ml kg⁻¹
infused over 30 minutes. The infusion started 15 minutes before the infusion of liquid into the stomach. Except for TLESRs and acid reflux episodes, basal LES pressure, duration of TLESR, latency time from start to first TLESR and swallowing rate were determined. Basal LES pressure was defined as the average pressure between the sleeve and the intragastric pressure during the 45 minute period. All swallow- and TLESR-related LES pressure changes were excluded. Acid exposure was expressed as the percentage of the 45 minute period during which esophageal pH was < 4. Reflux episodes were defined as a drop in pH > 1 unit within 5 seconds. The average pH in the 15 seconds following nadir pH should be < 4.

**Drugs**
All compounds were from Tocris, Bristol, U.K. and were dissolved in physiological saline (0.9% NaCl), except for diazepam, which was from Dumex-Alpharma, Copenhagen, Denmark, and delivered as a solution which was directly used at a dose of 3.5 µmol kg⁻¹ (1 mg kg⁻¹). The doses of muscimol which were tested were 8.2 µmol kg⁻¹ (1.0 mg kg⁻¹), 0.82 µmol kg⁻¹ (0.1 mg kg⁻¹), 0.27 µmol kg⁻¹ (0.033 mg kg⁻¹), and 0.082 µmol kg⁻¹ (0.01 mg kg⁻¹). A dose of 8.2 µmol kg⁻¹ (1.3 mg kg⁻¹) and 5.7 µmol kg⁻¹ (1 mg kg⁻¹) was used for isoguvacine and THIP, respectively.

**Calculations**
Each dog served as its own control. All parameters were calculated regarding the mean of five preceding control experiments. Data are presented as mean ± SEM. Statistical analysis was done using paired Student’s t-tests. A p value < 0.05 was regarded as statistically significant.

**Immunohistochemistry**

**Primary antibodies**
Different antibodies were used against different GABAₐ receptor subunits; mouse monoclonal anti-GABAₐ α (Biosite AB), mouse monoclonal anti-GABAₐ α1 (Chemicon International Inc and Boehringer), goat polyclonal anti-GABAₐ α2 and anti-GABAₐ α3 (Santa Cruz Biotechnology, Inc), mouse monoclonal anti-GABAₐ β (Chemicon International Inc) and rabbit polyclonal anti-GABAₐ γ2 (Chemicon International Inc).

**Immunolabeling procedure**
Fresh dog brain tissue and dog nodose ganglia were harvested and fixed in formaldehyde (formaline 10%) for at least 12 hours. The tissue was dehydrated in increasing concentrations of alcohol and xylene and then embedded in paraffin and sectioned using a microtome (Leica Microsystems) at 4 µm. Tissue sections were deparaffinized and rehydrated through xylene, graded ethanol series, distilled water and phosphate-buffered saline (PBS). Different pre-treatments were tried as antigen retriever; no pre-treatment, trypsin, high pressure boiling with Diva or Borg solution in a Decloaker (Biocare Medical, CA), or microwave boiling with a citrate buffer solution (0.01M, pH 6.0). Sections were then incubated for 5 minutes
in 3% hydrogen peroxidase (Merck, Germany) in distilled water, washed twice in PBS and preincubated with 10% normal donkey serum in PBS for 20 minutes at room temperature and then incubated with primary antibodies to one of the GABA<sub>A</sub> receptor subunits. The antibodies were diluted in ChemMate Antibody Diluent (Dako Cytomation, Denmark) 1:25 (α and γ) and 1:75 (β) and incubations were performed overnight at room temperature. The sections were washed three times in PBS and then incubated in biotinylated donkey-anti-rabbit, -mouse or -goat IgG (Jackson Immunoresearch, West Grove, PA, dilution 1:500) for 1 hour at room temperature. After washing the sections three times in PBS, the avidin-biotin complex (ABC) solution (Vectastain Elite Standard Kit (Vector Laboratories, Burlingame, CA) was applied for 45 minutes at room temperature. Following rinsing in PBS, immunoreactivity was visualized using 3-amino-9-ethylcarbazole for 10-20 minutes at room temperature. Finally, sections were counterstained with Mayer’s hematoxylin and coverslipped using a water based mounting media (Quick Mount, Daido Sangyo, Japan).

Negative controls included sections that were incubated in the presence of negative control immunoglobulin fraction from non-immunized rabbits or goats in the same dilution as the primary antibodies, or as antigen-antibody preabsorption experiments with the native antigen preincubated at 4 °C for 24 hours with the diluted antibody solution. Dog cerebellum was used as positive control.

Images were captured with the image program Picsara (Euromed Networks) from the light microscope using a Sony 3CCD video camera. The images were imported into Adobe Photoshop or Microsoft Photo Editor for minor adjustments of brightness, contrast and sharpness.

**RT-PCR**

Nodose ganglion and cerebellum (positive control) were taken from the same dog. RNA was prepared (TRIzol<sup>®</sup> Reagent, Invitrogen) and reverse transcribed into cDNA using oligo(dT)

<table>
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<th>Subunit</th>
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<tr>
<td>α1f</td>
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<td>18</td>
</tr>
<tr>
<td>α1r</td>
<td>5’-GAAAGCTATTTCTGACAGTCGGTC- 3’</td>
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<td>α1 probe</td>
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<tr>
<td>β2f</td>
<td>5’-GATGTTGAGAGTCCGGGAAAG- 3’</td>
<td>22</td>
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<td>β2r</td>
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<td>β2 probe</td>
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f = forward, r = reverse
and random primers (iScript™ cDNA Synthesis Kit, BIORAD). The cDNA was amplified by RT-PCR (Taqman® Universal PCR Master Mix, Applied Biosystem) using primer pairs and probes for GABA_A receptor subunits α1, β2, β3, and γ1 (Eurogentec S.A, Belgium). All primer sequences are listed in Table 1. Real time PCR was performed using a 7500 Real Time PCR System (Applied Biosystems). Product specificity of the PCR products was confirmed by agarose gel electrophoresis.

## Results

### Dog experiments

**TLESRs**

The GABA_A agonist muscimol produced a dose dependent inhibition of TLESRs ranging from 19 ± 8% at 0.082 µmol kg⁻¹ to 56 ± 17% at 8.2 µmol kg⁻¹ (n=6 for all doses). At maximal inhibition, the rate of TLESRs was significantly reduced from 11.0 ± 1.2 in control experiments to 5.5 ± 3.0 (p<0.05; Figure 1). Surprisingly, at the lowest and highest dose of muscimol no side effects occurred, while at the intermediate doses emesis occurred just after drug administration. No sedative effect was seen by muscimol. The two other GABA_A agonists evaluated, isoguvacine (8.2 µmol kg⁻¹) and THIP (5.7 µmol kg⁻¹), as well

![Figure 1.](image1.png)

**Figure 1.** The dose-dependent effect of muscimol on the rate of TLESRs. The effect is expressed in mean values (A) and in percentage of inhibition (B). At maximal inhibition, the rate of TLESRs was significantly reduced from 11.0 ± 1.2 in control experiments to 5.5 ± 3.0 in muscimol 8.2 µmol kg⁻¹ treated dogs, corresponding with an inhibition of 56% ± 17.5 (P<0.05).

![Figure 2.](image2.png)

**Figure 2.** Isoguvacine (A), THIP (B) and diazepam (C) have no significant effect on the rate of TLESRs.
as the GABA\(_A\) positive allosteric modulator diazepam (3.5 \(\mu\)mol kg\(^{-1}\)), had no major effects on TLESRs at the doses tested (isoguvacine 0 ± 12% change; THIP 15 ± 9% inhibition; diazepam 29 ± 12% inhibition; Figure 2), and were limited by emesis (isoguvacine and THIP) or restlessness and sedation (diazepam).

**Basal LES pressure**

Basal LES pressure was slightly increased by muscimol from 13 ± 13% at the lowest dose to 20 ± 10% at the highest dose (Figure 3), only statistically significant after muscimol 0.82 \(\mu\)mol kg\(^{-1}\) (controls 43 ± 4mmHg; muscimol 0.82 \(\mu\)mol kg\(^{-1}\) 49 ± 5mmHg (p<0.04)). The other compounds produced no significant effect on basal LES pressure.

![Figure 3. Effect of muscimol on basal LES pressure.](image)

The percentage increase varied from 13% ± 12.9 at the lowest dose to 20% ± 10.0 at the highest dose, only significant at 0.82 \(\mu\)mol kg\(^{-1}\) (13% ± 4.6; P<0.04).

**Reflux and other parameters**

Reflux episodes were significantly reduced by 25 ± 8.8% after 0.27 \(\mu\)mol kg\(^{-1}\) muscimol (controls 3.2 ± 0.37; muscimol 0.27 \(\mu\)mol kg\(^{-1}\) 2.4 ± 0.4 (p<0.03)). The other doses of muscimol, isoguvacine, THIP and diazepam showed no significant effect on reflux episodes (data not shown). Swallowing rate and latency time to the first TLESR were not significantly affected by any of the tested compounds (data not shown).

**Immunohistochemistry**

We were unable to detect any immunoreactivity, in either nodose ganglion or cerebellum, with the GABA\(_A\) \(\alpha\) and \(\alpha\)1 specific antibodies. Immunoreactivity of the GABA\(_A\) \(\alpha2\) and \(\alpha3\) subunits was observed scattered throughout the dog nodose ganglion, where it was localized in the cell bodies (Figure 4). The immunoreactivity in cerebellum (positive control tissue) of GABA\(_A\) \(\alpha2\) and \(\alpha3\) was found in the granular cell layer and in fibres of the white matter. The \(\beta\) subunit was intensely found in the granular cell layer of the cerebellum, but not in the nodose ganglion. GABA\(_A\) \(\gamma2\) immunoreactivity in the nodose ganglion was confined to fine varicose fibers surrounding the somata. This staining was not seen using the IgG negative controls (Figure 5). In the cerebellum, weak \(\gamma2\) immunoreactivity was found in the Purkinje cells and some staining was seen in endothelial cells of the brain and nodose ganglion.
**Figure 4.** (A) Photomicrograph showing the dog nodose ganglion with GABA $\alpha_3$ positive cell bodies. (B) Preabsorption control experiments for GABA $\alpha_3$. Scale bar = 50 $\mu$m. (See colour figures, page 157)

**Figure 5.** (A) Photomicrograph showing the dog nodose ganglion with positive GABA $\gamma_2$ fibers (arrows). (B) IgG control experiment for GABA $\gamma_2$. Scale bar = 20 $\mu$m. (See colour figures, page 157)

**Figure 6.** RT-PCR results with the different GABA receptor subunits expressed in dog nodose ganglion (A) and cerebellum as positive control (B). There is abundant expression of $\beta_3$, but lack of $\gamma_1$ expression in the dog nodose ganglion.
**RT-PCR**

The GABA$_A$ subunits $\alpha_1$, $\beta_2$ and $\beta_3$ but not $\gamma_1$ were detected in the dog nodose ganglion by PCR analysis. mRNA expression of these GABA$_A$ subunits in the dog cerebellum was used as positive control (Figure 6).

**Discussion**

In the present study, we investigated the role of GABA$_A$ agonists on the occurrence of TLESRs and the expression of GABA$_A$ subunit receptors in the nodose ganglion of the dog. This study showed a significant and dose dependent inhibition of TLESRs by the centrally acting GABA$_A$ agonist muscimol in the dog up to 56%. This suggests that the use of GABA$_A$ receptor agonists may be a novel strategy in the treatment of GERD. However, muscimol is less efficacious in the reduction of TLESRs compared to the GABA$_B$ receptor agonist baclofen, which previously showed a reduction of postprandial TLESRs in dogs up to 90%.\(^{25}\) The maximal effect of baclofen has been reproduced in several independent studies in our laboratory (Lehmann et al., manuscript in preparation and unpublished) so it was not deemed necessary to include a separate baclofen dose group in the current study. If the difference between muscimol and baclofen in terms of efficacy can be generalised to their respective targets, it appears that GABA$_B$ receptor agonists would offer a more attractive option than GABA$_A$ receptor agonists in the quest for novel drugs for treatment of GERD.

Muscimol has been reported to inhibit TLESRs in ferrets, but only at a dose (10 µmol kg$^{-1}$) which induced sedation.\(^{8}\) In the current experiments, reduction of TLESRs could not be secondary to sedation since this side-effect was not seen at the doses used. In contrast to the effect of muscimol, the less potent GABA$_A$ agonist THIP $^{26}$ and the peripherally restricted GABA$_A$ agonist isoguvacine $^{27}$ showed no effect on the occurrence of TLESRs. The emetogenic effects of both THIP and isoguvacine limited further studies with higher doses. THIP has been administered in baboons at a dose ranging from 0.25 - 8.0 mg kg$^{-1}$ iv.$^{28}$ However, in our study emesis occurred at doses higher than 1 mg kg$^{-1}$, even in the absence of intubation and administration of a high gastric load. THIP has potent anxiolytic and analgesic properties in man but is known to have approximately 8 - 10 times lower affinity for GABA$_A$ receptors compared to muscimol.$^{28-30}$ Isoguvacine has poor central nervous system penetration $^{31}$ and acts as a peripherally restricted GABA$_A$ agonist.$^{27}$ However, the hypothesis that a peripherally restricted agonist would augment or reduce the number of TLESRs by activating or inhibiting vagal afferents could not be confirmed in our study. This may suggest that a central but not peripheral site of action of GABA$_A$ agonists plays a critical role in the control of TLESRs. Although there was a tendency towards inhibition of TLESRs, the positive GABA$_A$ modulator diazepam had no statistically significant effect. In analogy with mice devoid of the $\gamma_2$-subunit, the lack of $\gamma_2$-subunit expression in vagal afferents in our study may render them insensitive to benzodiazepines $^{15,20}$, and therefore an effect of diazepam on the rate of TLESRs through binding to vagal afferent terminals might be not anticipated. In addition, general sedation is thought to reduce the number
of TLESRs. In our study, however, sedation occurred with diazepam, but no significant reduction in TLESRs was seen. It should be noted that the onset of sedation in the dogs followed a period of restless behaviour, and any effect of sedation on TLESRs could be masked. However, TLESRs were seen throughout the total study time after diazepam, and they peaked during the first 20 minutes as in control experiments. It would be important to assess whether there is a GABA<sub>A</sub> tone controlling TLESR by using receptor antagonists. However, GABA<sub>A</sub> receptor antagonists can trigger epileptic seizures, and ethical barriers prevent such experiments in dogs.

Muscimol has no effect on basal LES pressure in ferrets but it slightly increased LES pressure in dogs. This effect is likely to be mediated in the hindbrain since muscimol has been shown to elevate basal LES pressure in cats upon hindbrain microinjection. In the management of GERD, stimulation of LES pressure is considered to have a beneficial effect in those patients who display long periods of low or absent LES pressure, which possibly promote reflux. There were discrepancies between the effect of muscimol on TLESRs and acid reflux episodes since the two highest doses significantly attenuated TLESRs but not reflux. However, this difference is probably more apparent than real as the number of reflux episodes was quite low, and changes were therefore difficult to identify. Also, the pHmetric method used fails to detect superimposed reflux, i.e., reflux occurring shortly after a preceding reflux episode which already has acidified the distal esophagus.

The distribution of various GABA<sub>A</sub> subunits in the NG has to our knowledge not been described before in any species including the dog. Interestingly, GABA itself has been detected immunocytochemically in the feline NG. Its function there, if any, is obscure. Immunohistochemical studies on GABA<sub>A</sub> subunits in peripheral ganglia have been performed in a few cases. For instance, in situ hybridisation showed expression of α2, β2 and γ2 in dorsal root ganglia of rats and all GABA<sub>A</sub> receptors subunits were found in rat spinal ganglion using immunohistochemistry. Although the present study focused on another peripheral ganglion, the nodose ganglion, literature data support the presence of GABA<sub>A</sub> receptor subunits in peripheral ganglion cells. We showed immunoreactivity with the α2 and 3 subunits in cell bodies of the dog nodose ganglion, and with γ2 in pericellular fibers. In line with this, additional RT-PCR showed α1, β2 and 3, but no γ1 subunit expression in the dog nodose ganglion. We were unable to detect any immunoreaction with the α and α1 specific antibodies in our paraffin embedded material. This is surprising, as the predominant GABA<sub>A</sub> receptor subunit combination throughout the brain is composed of α1β2/3γ2. The fact that most other studies have been performed on frozen material could explain this difference in observed immunoreactivity. Presence of the α1 subunit is nevertheless indicated with RT-PCR, and it can therefore be speculated that several α subunits are present in the NG, together with β subunits, especially β3 according to the RT-PCR experiments. However, our immunohistochemical studies did not demonstrate convincing β2 staining in the nodose ganglion. A possible explanation for this could be the fact that the used antibodies were not directed against dog GABA<sub>A</sub>. Interestingly is the immunohistochemical finding of the γ2 subunit in pericellular nerve fibers rather than in the ganglion cells of the NG. Previous studies have demonstrated the presence of such pericellular fibers in the nodose ganglion.
of the monkey, rabbit and pigeon.\textsuperscript{41, 42} Also GABA\textsubscript{B} receptor 1a immunoreactivity has been found on fibers surrounding the nerve cell bodies in guinea pig nodose ganglion.\textsuperscript{23} In conclusion, the present study revealed expression of GABA\textsubscript{A} receptor subunits in the dog nodose ganglion. In addition, we showed involvment of GABA\textsubscript{A} receptors in the control of TLESRs. The GABA\textsubscript{A} receptor agonist muscimol reduced the rate of TLESRs by 19-56% depending on dose. The potential of GABA\textsubscript{A} agonists as inhibitors of TLESRs and therefore as future anti-reflux agents depends on their therapeutic margin. While the currently available GABA\textsubscript{A}-stimulating drugs registered for other indications carry a side-effect profile not compatible with their use in GERD, emerging GABA\textsubscript{A} subunit selective compounds \textsuperscript{43} may be useful in this context. Therefore, further studies are warranted in which the effects of GABA\textsubscript{A} subtype selective compounds on TLESRs are assessed.

\textbf{Reference List}


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