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Inhibitory effects of $GABA_B$ receptor agonists on swallowing in the dog

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Abstract

The effects of the $GABA_B$ receptor agonists baclofen (1.4 and 7 μmol/kg i.v.) and CGP 44532 ([(2*S*)-3-amino-2-hydroxypropyl]methyl phosphinic acid], 0.2 and 0.7 μmol/kg i.v.) on transient lower esophageal sphincter relaxations and spontaneous and pharyngeally stimulated swallowing were investigated in conscious dogs. Both compounds inhibited transient lower esophageal sphincter relaxations dose-dependently, CGP 44532 being approximately fivefold more potent. In experiments designed to measure transient lower esophageal sphincter relaxations, spontaneous swallowing was suppressed by both compounds. When swallowing was evoked by intrapharyngeal water injection, both baclofen and CGP 44532 reduced the occurrence of primary peristalsis. It is concluded that centrally acting $GABA_B$ receptor agonists inhibit spontaneous and stimulated swallowing probably through an action in the central pattern generator for swallowing.

Keywords: $GABA_B$ receptor; Swallowing; Esophageal sphincter relaxation, transient, lower; Baclofen

1. Introduction

The major inhibitory neurotransmitter γ-aminobutyric acid (GABA) acts on two receptors, the ionotropic pentameric $GABA_A$ receptor and the metabotropic heterodimeric $GABA_B$ receptor. The $GABA_B$ receptor was cloned recently and found to be the product of two separate genes, $GABA_B$ receptors R1 and R2 (Billinton et al., 2001). Activation of the $GABA_B$ receptor produces via coupling to G proteins, inhibition of voltage-dependent Ca^{2+} channels, opening of inwardly rectifying K^+ channels and inhibition of adenylate cyclase (Billinton et al., 2001).

The $GABA_B$ receptor was first defined in peripheral tissues (Bowery et al., 1980), but most investigations have been directed towards the role of central $GABA_B$ receptors. $GABA_B$ R1 knock-out mice were recently generated and they display a phenotype consistent with changes in central nervous system (CNS) function (Schuler et al., 2001; Prosser et al., 2001). Likewise, most interest in potential therapeutic utility of $GABA_B$ receptor agonists has focussed on indications such as pain, epilepsy and cognitive disorders. Relatively little effort has been made to discover potentially therapeutic effects of $GABA_B$ receptor agonists in diseases which have a typical peripheral manifestation but which may have central pathogenetic components. We have found that the prototypic $GABA_B$ receptor agonist baclofen inhibits transient lower esophageal sphincter relaxation in dogs (Lehmann et al., 1999), a finding which has been replicated in ferrets (Blackshaw et al., 1999) and healthy humans (Lidums et al., 2000), and most recently in gastroesophageal reflux disease patients (Zhang et al., 2002). This observation may have major therapeutic implications since transient lower esophageal sphincter relaxations are the chief cause of gastroesophageal reflux (Dodds et al., 1982).

The effect of $GABA_B$ receptor agonists on transient lower esophageal sphincter relaxations seems to involve vagal mechanosensitive afferents signalling gastric distension (Blackshaw, 2001; Partosoedarso et al., 2001). In addition, there appears to be an inhibitory effect of $GABA_B$ receptor agonists at the level of the dorsal vagal complex (McDermott et al., 2001; Partosoedarso et al., 2001). Effects have also been demonstrated on vagal motoneurons supplying the lower esophageal sphincter (Smid and Blackshaw, 2000), but the inhibitory enteric neurons innervating the smooth muscle cells of the lower esophageal sphincter, as well as the muscle cells themselves, can be excluded as targets for $GABA_B$ receptor agonists (Blackshaw et al., 2000).

In our previous studies on dogs (Lehmann et al., 1999), we noted without doing any formal analysis that the

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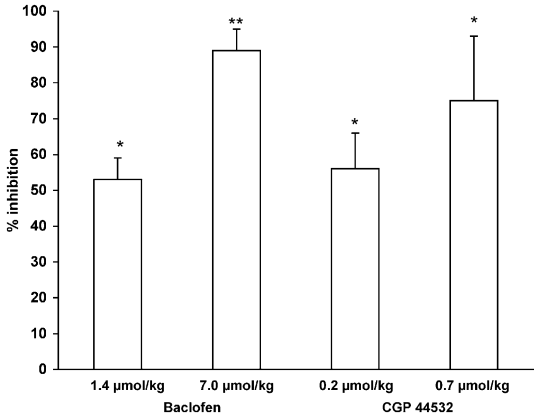

Fig. 1. Effects of baclofen and CGP 44532 on transient lower esophageal sphincter relaxations in dogs. The compounds were administered intravenously 10 min before intragastric infusion of liquid nutrient followed by insufflation of air. The total time of infusion and insufflation was 45 min. $n=3$ in the CGP 44532 groups, 6 in the baclofen 1.4 µmol/kg group and 5 in the baclofen 7 µmol/kg group. $*P<0.05$, $**P<0.01$ vs. control (Student's paired t-test). Control incidence of transient lower esophageal sphincter relaxation was $6.2 \pm 0.5/45$ min ($n=9$).

swallowing incidence after administration of baclofen was markedly reduced. In the experimental setting used, most swallows were triggered by transient lower esophageal sphincter relaxations. It is, therefore, uncertain if the effect of $GABA_B$ receptor agonists were secondary to inhibition of transient lower esophageal sphincter relaxations or to direct effects. In the present work, we studied the effects on spontaneous and pharyngeally induced swallowing of baclofen and GCP 44532 [(2*S*)-3-amino-2-hydroxypropyl]methyl phosphinic acid], another $GABA_B$ receptor agonist (Froestl et al., 1995; Ong et al., 2001) which in ferrets has been found to selectively affect transient lower esophageal sphincter relaxation but not swallowing in contrast to baclofen (Blackshaw et al., 1999).

2. Materials and methods

The studies were performed on adult Labrador retrievers and beagles previously equipped with an esophagostomy for intubation (Lehmann et al., 1999). To compare the effects of the two $GABA_B$ receptor agonists on transient lower esophageal sphincter relaxations and spontaneous swallows, baclofen (1.4 and 7 µmol/kg) and CGP 44532 (0.2 and 0.7 µmol/kg) were administered intravenously during 2 min at 0.5 ml/kg. Transient lower esophageal sphincter relaxations were stimulated with a combination of a liquid nutrient and free air insufflation and measured using Dentsleeve manometry (Lehmann et al., 1999). Transient lower esophageal sphincter relaxations were defined and quantitated according to the criteria described by Lehmann et al. (1999). Swallows accompanied by successful peristalsis were defined according to Stakeberg and Lehmann (1999).

In separate experiments, dogs which had been fasting for some 18 h were intubated with a Dentsleeve multilumen assembly and two pharyngeal catheters (one for water injection and one for pressure recording). Water at room temperature was injected through the pharyngeal catheter at an approximate rate of 0.2 ml/s and the injection was discontinued when the dog swallowed. An effective volume producing swallowing after at least 75% of the injections was established, and 10–15 injections separated by 1 min were then given. Occasionally, this time had to be extended slightly because of multiple swallowing. Placebo or active compound was then given through a foreleg vein at 0.5 ml/kg during two min. Ten minutes later, 10–15 injections were made with the volume established in control experiments, and the number of swallows triggering successful peristalsis was analysed and expressed as percent of the number of injections. All experiments were approved by the Ethical Committee for Animal Experiments of the Göteborg region.

Racemic baclofen was purchased from RBI, Natick, MA, USA, and CGP 44532 was synthesised by S. von Unge, AstraZeneca R&D Mölndal, Sweden. The compounds were dissolved in 0.9% NaCl immediately before use.

3. Results

The incidence of transient lower esophageal sphincter relaxation in the placebo-controlled experiments was $6.2 \pm 0.5/45$ min (mean ± S.E.M., $n=9$). Both baclofen and CGP 44532 produced a dose-dependent inhibition of transient lower esophageal sphincter relaxation (Fig. 1). As only two doses of each compound were administered, ED_{50} values could not be calculated but CGP 44532 was approximately 5–10 times more potent than baclofen (Fig. 1). The effects of CGP 44532 were qualitatively similar to baclofen in all aspects. For instance, the compound reduced the number of transient lower esophageal sphincter relaxations but not their appearance (data not shown). Spontaneous swallowing was reduced by both baclofen and CGP 44532 (Fig. 2) although the inhibition produced by the latter failed

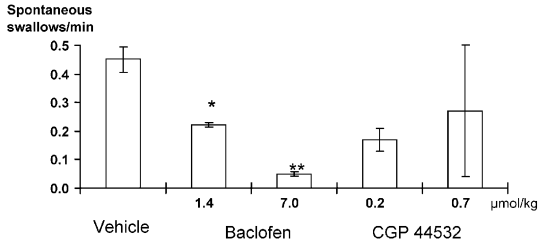

Fig. 2. Effects of baclofen and CGP 44532 on spontaneous swallowing in dogs. The number of swallows was quantitated in the experiments in which transient lower esophageal sphincter relaxations were measured. $*P<0.05$,$**P<0.01$ vs. control (Student's paired t-test). The number of experiments is given in legend of Fig. 1. Note that the vehicle group represent pooled data from all experiments.

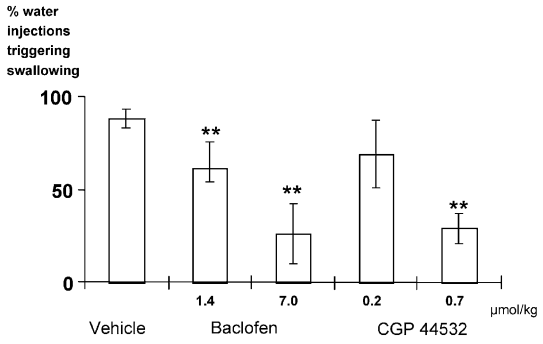

Fig. 3. Effects of baclofen and CGP 44532 on stimulated swallowing in dogs. Water was injected 10–15 times in the pharynx at a volume producing swallowing in 75–80% of the cases. The compounds were then administered, and the water injections were repeated after 10 min. ** $P<0.01$ vs. control, Student's paired t-test, $n=8$ in the baclofen groups and 6 in the CGP 44532 groups. Note that the vehicle group represent pooled data from all experiments.

to reach statistical significance due to the relatively low number of experiments. While there was a clear dose-dependency for the effects of baclofen, it was not obvious for CGP 44532. The reason for this was that one of the dogs that was given CGP 44532 at 0.7 μmol/kg of had several bouts of multiple swallowing. No behavioural side effects were seen after administration of the compounds.

Injection of water reproducibly triggered swallowing at an average injection volume of 4.4 ± 0.4 ml (mean ± S.E.M.). While baclofen and CGP 44532 reduced the number of swallows followed by successful peristalsis (Fig. 3), the pharyngeal contraction seemed less affected. Both baclofen and CGP 44532 dose-dependently inhibited the water-induced swallowing reflex with a potency comparable to their effect on transient lower esophageal sphincter relaxations.

4. Discussion

Suppression of spontaneous swallowing after baclofen administration reported here agrees with findings made in humans (Lidums et al., 2000; Zhang et al., 2002) and ferrets (Blackshaw et al., 1999). The present study suggests that baclofen and the more potent $GABA_B$ receptor agonist CGP 44532 both inhibit spontaneous swallowing in experiments designed to quantitate transient lower esophageal sphincter relaxation. CGP 44532 was 5–10 times more potent than baclofen in inhibiting transient lower esophageal sphincter relaxations. This is compatible with studies on recombinant human $GABA_{B(1a,2)}$ (J. Mattsson, unpublished data), rat $GABA_B$ autoreceptors (Ong et al., 2001), as well as on muscle relaxation in rats (Froestl et al., 1995). However, it differs substantially from the relatively small disparities in potencies of baclofen and CGP 44532 on rat CNS hetero-receptors (Ong et al., 2001). The present observations contrast previous findings in the ferret in which only baclofen but not CGP 44532 inhibited spontaneous swallowing at doses that effectively blocked transient lower esophageal sphincter relaxations (Blackshaw et al., 1999).

The attenuated swallowing incidence was not solely due to reduction in transient lower esophageal sphincter relaxation-triggered swallowing since swallowing induced by pharyngeal water injection also was inhibited by the $GABA_B$ receptor agonists. The site of action of the compounds was not studied but it is appears that the inhibition occurred at the level of the brain stem. This suggestion is based on the finding that the pharyngeal contractile response to water stimulation seemed largely unperturbed, but the esophageal peristaltic response was inhibited by the $GABA_B$ receptor agonists. A possible explanation for this is that while the pharyngopharyngeal reflex arc may be unaffected, the transmission between the pharyngeal afferents and the esophageal motoneurons is inhibited by $GABA_B$ receptor agonists. Both the central subnucleus of the nucleus of the solitary tract, as well as the nucleus ambiguus, the premotor and motor nuclei of the esophagus, respectively, are candidate targets for the effect of $GABA_B$ receptor agonists since both are richly endowed with $GABA_B$ receptors (Margeta-Mitrovic et al., 1999; McDermott et al., 2001). It is not known if the receptors in these regions are pre-or postsynaptic or both, but it is noteworthy that presynaptic $GABA_B$ receptors in the brain stem are considerably more sensitive to baclofen than the postsynaptic receptors (Brooks et al., 1992).

$GABA_B$ receptor agonists such as baclofen are effective inhibitors of transient lower esophageal sphincter relaxations, and the site of action appears to reside both at the peripheral endings of gastric vagal mechanoreceptors, as well as centrally, presumably through inhibition of transmitter release from the vagal endings in the nucleus of the solitary tract (Blackshaw, 2001). It has been directly demonstrated that baclofen also can inhibit gastric neurons of the dorsal motor nucleus of the vagus at the cell body level (Browning and Travagli, 2001) and at the level of their peripheral terminals (McDermott et al., 2001). It is, however, questionable if baclofen and CGP 44532 act on dorsal motor nucleus of the vagus neurons to inhibit transient lower esophageal sphincter relaxations since neither reductions in swallow-induced relaxations of the lower esophageal sphincter nor incomplete transient lower esophageal sphincter relaxations have been described after injection of baclofen. The mechanism of action of $GABA_B$ receptor agonists on evoked swallowing, thus, resembles that proposed for inhibition of transient lower esophageal sphincter relaxation in that it probably takes place at a site more proximal in the circuitry than the motoneurons.

Agents that inhibit transient lower esophageal sphincter relaxations such as baclofen, atropine and morphine also reduce spontaneous swallowing (Mittal et al., 1995; Penagini and Bianchi, 1997; Lidums et al., 2000; Zhang et al., 2002). Since the lower esophageal sphincter relaxation seen during both swallowing and transient lower esophageal

sphincter relaxation may be mediated through the same neuronal pool of the dorsal motor nucleus of the vagus, the site of action of these compounds is probably at the level of integration of lower esophageal sphincter relaxation and esophageal activation.

Baclofen has been shown to inhibit vagal afferent sensitivity with some selectivity (Partosoedarso et al., 2001). However, regardless of site of action, baclofen can inhibit other vagally controlled functions as well (Bousquet et al., 1982). The present data suggest that $GABA_B$ receptor agonists also can inhibit the pharyngo-esophageal swallowing reflex, possibly through a CNS site of action. Peripherally acting $GABA_B$ receptor agonists may consequently not only lack effects on alertness and other important mental functions but also be void of effects on swallowing patterns.

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