Electrophysiological, biochemical, and functional release studies suggest that γ-aminobutyric acid (GABA)B receptors are pharmacologically heterogeneous. Analysis of GABAergic twitch contractions and of acetylcholine release in guinea pig ileum (Bowery et al., 1981; Kaplita et al., 1982; Giotti et al., 1983; Kleinrok and Kilhinger, 1983; Ong and Kerr, 1983; Taniyama et al., 1992, 1996). Presynaptic release-inhibiting GABAergic receptors display clear pharmacological diversity, based on their differential sensitivity to (−)-baclofen and to various selective GABA B receptor antagonists (Bonanno and Raiteri, 1993).

Activation of GABA B receptors was shown to mediate inhibition of electrically evoked cholinergic twitch contractions and of acetylcholine release in guinea pig ileum (Bowery et al., 1981; Kaplita et al., 1982; Giotti et al., 1983; Kleinrok and Kilhinger, 1983; Ong and Kerr, 1983; Taniyama et al., 1992, 1996). In analogy with the guinea pig, activation of GABA B receptors in human small intestine was reported to inhibit longitudinal muscle motility through an action on cholinergic neurons (Gentilini et al., 1992). The finding that baclofen could inhibit the tetrodotoxin-insensitive release of [3H]acetylcholine evoked by high K+ (Taniyama et al., 1992, 1996), together with previous electrophysiological evidence (Cherubini and North, 1984), suggests that GABA B receptors are located on cholinergic nerve endings of the myenteric plexus.

The GABA B receptors inhibiting the electrically evoked cholinergic twitch contraction in guinea pig ileum were previously reported to be phaclofen- and CGP 35348-sensitive (Kerr et al., 1987; Ong et al., 1994). However, multiple phaclofen- and CGP 35348-sensitive GABA B receptors were found to exist in the rat central nervous system (CNS; Gemignani et al., 1994; Bonanno et al., 1999), which justifies further pharmacological characterization of the intestinal GABA B receptors.

In the present work, we determined the pharmacological profile of the GABA B receptor inhibiting the electrically evoked cholinergic twitch contraction from myenteric plexus-longitudinal muscle (MP-LM) preparations of guinea pig ileum by using a series of ligands (CGP 52432 and CGP 47656; Gemignani et al., 1994; CGP 36742; Bonanno et al., 1999) able to distinguish receptor subtypes within the phaclofen- and CGP 35348-sensitive GABA B receptor group. In addition, CGP 56999, a potent antagonist supporting heterogeneity of presynaptic GABA B receptors in the rat spinal cord (Teoh et al., 1996), was used.

**Materials and Methods**

**Animals and Tissue Preparation.** Male guinea pigs, weighing 350 to 450 g, were sacrificed by cervical dislocation. Terminal ileum was removed after the 10 cm nearest to the ileocecal junction had been removed.
been discarded and strips of myenteric plexus with the longitudinal muscle attached (MP-LM) were prepared according to Paton and Vizi (1969).

**Single Twitch Stimulation.** Four MP-LM strips (3–4 cm in length) from each animal were mounted in separate 3-ml organ baths perfused with Tyrode’s solution of the following composition: 136.9 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl2, 1.04 mM MgCl2, 11.9 mM NaHCO3, 0.4 mM NaH2PO4, and 10 mM glucose, at 37°C, and continuously oxygenated (95% O2, 5% CO2, pH 7.2–7.4). An initial tension of 0.5 g was applied, and the longitudinal muscle activity was recorded through an isometric force transducer. After 120-min perfusion at 1 ml/min, longitudinal muscle repetitive twitch contractions (developing tension of 1.2–2.3 g) were evoked by field stimulation (alternating rectangular pulses, 0.1 Hz, 1-ms duration, 550–600 mA) delivered from two platinum electrodes. The use of submaximal voltage was a precaution against the effects of drugs being masked by supramaximal pulses (see Fosbraey and Johnson, 1980).

The effects of drugs were measured as percent variation of the cholinergic twitch contraction height. To avoid desensitization (Bowery et al., 1981; Ong and Kerr, 1983), agonists were applied at 30-min intervals; when the response to any agonist concentration had reached a maximum, tissues were washed and perfused at 1 ml/min for 20 min. Two complete concentration-response curves were conducted in the same tissue. When the effects of antagonists on the agonist inhibition of twitch contraction were evaluated, after a first concentration-response curve for the agonist was completed in standard medium, a second concentration-response curve was made in standard medium (control) or in the presence of the antagonists (one antagonist concentration only per tissue). Antagonists were added to the perfusion medium 20 min before their effects were evaluated. When the effects of adding CGP 47656 on (−)-baclofen were evaluated, after a first concentration-response curve for (−)-baclofen was completed, a second concentration-response curve was made for (−)-baclofen alone (control) or by adding (−)-baclofen together with CGP 47656 at various concentrations. In each experiment, a preparation was run as control; in the experimental conditions used in control preparations, the agonist EC50 value in the second curve did not significantly differ from the EC50 value in the first curve. Data obtained in first (agonists alone) and second (in the presence of antagonists) curves are given in the figures. In preliminary experiments, the effects of compounds on longitudinal muscle contractile responses to acetylcholine were evaluated in MP-LM preparations.

**Data Analysis.** Concentration-response curves for agonist inhibition of the twitch contraction were analyzed by a four-parameter logistic function analysis (SigmaPlot software). pD2 values of agonists were measured as −log EC50 values (EC50 is the concentration producing 50% of the drug maximum effect). The pA2 value of CGP 52432 was measured according to Arunlakshana and Schild (1959); when the concentration range (equal to or less than 10-fold) of the antagonists precluded construction of a Schild plot to provide true pA2 values, apparent pA2 values were determined using the relation pA2 = log (EC50 ratio − 1) − log (B) in the presence of at least two different antagonist concentrations (B). The pKb value for CGP 47656 antagonism of (−)-baclofen was estimated from agonist dose ratios producing half-maximum responses in accordance with the model for partial agonism: pKb = log (EC50 ratio − 1) − log (B) in the presence of at least two different CGP 47656 concentrations (B). Mean ± S.E. values of determination in n separate experiments are indicated throughout. The statistical significance of the differences between mean values was assessed by the Student’s t test. A probability level of P < .05 was taken as statistically significant.

**Drugs.** Acetylcholine hydrochloride, GABA, and (−)-bicuculline methobromide were purchased from Sigma Chemical Co. (St. Louis, MO). Phaclofen was purchased from Tocris Cookson (Bristol, UK). (−)-Baclofen, 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP 35348), 3-aminopropyl-n-butyl phosphinic acid (CGP 36742), 9-amino-3-propyl-difluoromethylphosphinic acid (CGP 47656), [3-[1(R)-3-carboxyphenyl]ethylamino]-2-(S)-hydroxy-propyl]cychoexyl-methyl-phosphinic acid (CGP 56999) were gifts from Novartis (Basel, Switzerland). All compounds were dissolved in distilled water or Tyrode’s solution.

**Results**

**Effects of GABA Receptor Agonists on Cholinergic Twitch Contraction.** GABA and (−)-baclofen inhibited the twitch contraction height in the presence of drugs was measured as percentage of the contraction before the addition of the drugs (control). Each point represents the mean ± S.E. of 6 to 32 determinations in separate experiments.

**Fig. 1.** Effects of GABA ( Curve), (−)-baclofen ( ), or CGP 47656 ( ) on cholinergic twitch contractions in guinea pig ileum MP-LM preparations. Twitch contraction height in the presence of drugs was measured as percentage of the contraction before the addition of the drugs (control). Each point represents the mean ± S.E. of three to six separate determinations.

**Fig. 2.** Effects of (−)-baclofen and CGP 47656 on cholinergic twitch contractions in guinea pig ileum MP-LM. Concentration-response relationships for (−)-baclofen alone (●) or added together with 3 μM (○), 30 μM (▼), or 300 μM (△) CGP 47656 are shown. Twitch contraction height in the presence of drugs was measured as percentage of the contraction before the addition of the drugs (control). Each point represents the mean ± S.E. of three to six separate determinations.
cholinergic twitch contraction by a maximum of about 80%, exhibiting quite close potencies (pD2 for GABA = 5.70 ± 0.08, n = 9; pD2 for (−)-baclofen: 5.33 ± 0.05, n = 32; Fig. 1). GABA, at the concentration of 30 μM, induced transient, bicuculline-sensitive contraction superimposed on the repetitive twitch (data not shown), before inhibition of twitch contraction.

The compound CGP 47656 reduced the cholinergic twitch contraction by a maximum of 35.2 ± 3.7% (mean of n = 24 experiments), exhibiting a pD2 value of 5.42 ± 0.10 (n = 24; Fig. 1). The inhibition of the contraction produced by 30 μM CGP 47656 (32.8 ± 4.5%; n = 8) was abolished in the presence of various GABA<sub>A</sub> receptor antagonists, including phaclofen (10 mM), CGP 35348 (300 μM), CGP 52432 (3 μM), and CGP 36742 (30 μM). Occasionally, CGP 47656 (300 μM) elicited a transient bicuculline (30 μM)-sensitive contraction superimposed on the repetitive twitch before inhibition of twitch contraction. Up to the highest concentrations used, CGP 47656 had no effect on the contractile responses to acetylcholine (0.01–0.1 μM; data not shown).

In the presence of the GABA<sub>A</sub> receptor antagonist bicuculline (30 μM), the potencies of GABA, (−)-baclofen, and CGP 47656 in inhibiting twitch contraction remained unaffected (pD2 for GABA = 5.50 ± 0.10, n = 3; pD2 for (−)-baclofen = 5.17 ± 0.07, n = 18; pD2 for CGP 47656 = 5.20 ± 0.12, n = 4).

To better understand the effect of CGP 47656, apparently characteristic of a partial agonist, concentration-response curves for (−)-baclofen were constructed in the presence of varying concentrations (3, 30, or 300 μM) of CGP 47656. The results, illustrated in Fig. 2, show that the effects of low concentrations of (−)-baclofen were potentiated by CGP 47656, whereas the effects of higher concentrations of (−)-baclofen were depressed by CGP 47656; the estimated CGP 47656 pK<sub>B</sub> value was 5.62 ± 0.05 (n = 4).

Blockade of (−)-Baclofen Effect by GABA Receptor Antagonists. The GABA<sub>A</sub> receptor antagonists phaclofen, CGP 35348, CGP 36742, and CGP 52432 shifted to the right the concentration-response curve of (−)-baclofen in a way compatible with competitive antagonism (Fig. 3). In confirmation of previous data (Kerr et al., 1990b; Ong et al., 1994), CGP 35348 was more potent than phaclofen, with apparent
pA2 values for CGP 35348 and phaclofen of 5.02 ± 0.09 (n = 4) and 3.90 ± 0.06 (n = 3), respectively. The compound CGP 36742 was almost equipotent with CGP 35348 (apparent pA2 = 4.88 ± 0.10, n = 6), whereas CGP 52432 exhibited the highest potency (pA2 = 7.82 ± 0.08, n = 6).

The compound CGP 56999 potently inhibited the (−)-baclofen effect; however, the shifts of the (−)-baclofen concentration-response curve produced by this drug are suggestive of a noncompetitive antagonism (Fig. 4).

The GABAB receptor antagonists, at the highest concentrations used, slightly increased the twitch contraction height (by about 10%) leaving unaltered the longitudinal muscle tone, with the exception of phaclofen, which, at the concentration of 1 mM, evoked a transient, bicuculline (30 μM)-sensitive contraction superimposed on twitch response. None of the antagonists tested affected the contractile responses to acetylcholine; the acetylcholine EC50 values in the presence of the highest antagonist concentrations used ranged from 53 ± 6.7 to 43 ± 5.9 nM (n = 3), which did not differ from 47 ± 5.2 nM (n = 6), the EC50 value in standard medium.

Discussion

GABA and (−)-baclofen inhibited cholinergic twitch contraction in guinea pig ileum MP-LM preparations, exhibiting potencies similar to those found for the two agonists in previous studies with peripheral tissues (Bower et al., 1981; Ong and Kerr, 1983; Ong et al., 1994). Moreover, in keeping with published reports (Kerr et al., 1987, 1990b; Ong et al., 1994), the receptor acted on by (−)-baclofen was sensitive to both phaclofen and CGP 35348.

Studies performed in our laboratory with CNS nerve terminals have shown that phaclofen- and CGP 35348-sensitive GABAB receptors are, however, heterogeneous (Gemignani et al., 1994; Bonanno et al., 1999). In particular, based on the differential sensitivities to other GABAB-selective ligands, including CGP 47656, CGP 52432, and CGP 36742 (see Table 1), two pharmacologically distinct subtypes within the phaclofen- and CGP 35348-sensitive group of GABAB receptors were found to exist in the rat cerebral cortex, where they are sited, respectively, on somatostatin (SRIF)- and cholecystokinin (CCK)-releasing axon terminals and mediate inhibition of neuropeptide release.

The results of the present work show that the pharmacological profile of the GABAB receptor inhibiting cholinergic twitch contraction in guinea pig ileum MP-LM can be clearly distinguished from those of the GABAB receptors regulating peptide release in the rat cerebrocortex. The intestinal receptor exhibits the highest apparent affinity for CGP 52432 and much lower affinity for CGP 36742, whereas the GABAB receptor present on SRIF-releasing terminals in the rat cortex shows the highest affinity for CGP 36742. Moreover, CGP 52432 could block the guinea pig intestinal GABAB receptor with a potency much higher than CGP 35348, whereas the two antagonists displayed identical potency at the rat cortical receptor involved in the release of SRIF. The pharmacology of the guinea pig ileum receptor does not resemble that of the GABAB receptor regulating CCK release in rat neocortex either. In fact, CGP 36742 was ineffective at the latter receptor up to 100 μM (Bonanno et al., 1999); in contrast, CGP 36742 was an antagonist as potent as CGP 35348 at the guinea pig ileum receptor. Moreover, although arguments based on agonist effects are of limited value to receptor characterization, CGP 47656 up to 100 μM, which is ineffective as an agonist or antagonist at the GABAB receptor regulating CCK release (Gemignani et al., 1994), inhibited twitch contractions with potency comparable to that of (−)-baclofen, although with lower efficacy (see Fig. 1). Because modulation of cholinergic nerve activity could be of therapeutical value in regulation of gut motility (see Taniyama et al., 1996), compounds acting as partial agonists/antagonists of the GABAB receptor controlling intestinal cholinergic motor events could be of interest in treating gut motility disorders.

In human cerebral cortex, SRIF- or CCK-releasing nerve terminals are equipped with GABAB receptors at which the compound CGP 47656 behaved as a full agonist and the antagonist CGP 52432 exhibited higher potency with respect to CGP 35348 (Bonanno et al., 1996; Raiteri et al., 1996; see Table 1). Interestingly, the compound CGP 36742 was recently found to distinguish between these two receptors in human cerebral cortex, as it does in the rat, being an antagonist at the receptor on SRIF-releasing nerve terminals but inactive (up to 100 μM) at the receptor regulating CCK release (Bonanno et al., 1999; see Table 1). Thus, the pharmacology of the GABAB receptor inhibiting cholinergic twitch contraction in guinea pig ileum resembles that of the receptor regulating SRIF release in human cerebral cortex; however, further studies with even more selective and additional ligands would be required before a firm conclusion can be established. The lower efficacy of CGP 47656 at the guinea pig ileum receptor could simply depend on a lower efficacy of receptor coupling in the ileum. Similarly, 3-aminopropylphosphonic acid and (R)-β-phenyl-GABA were full agonists at rat CNS GABAB receptors but partial agonists at the GABAB receptors in guinea pig ileum myenteric plexus.
found in peripheral systems where GABA_B receptor-mediated functions have been described by several authors (for reviews see Bowery, 1993; Chapman et al., 1993; Taniyama et al., 1996).

The GABA_B receptor present in the guinea pig ileum characterized in the present work is pharmacologically distinct from any of the several subtypes previously identified in the rat CNS (see Bonanno et al., 1999) and displays similarity to the GABA_B receptor regulating SRIF release in human neocortex.

**References**


