0026-895X/94/030558-05$03.00/0

MOLECULAR PHARMACOLOGY, 46:558–562, 1994.

Pharmacological Discrimination Between γ-Aminobutyric Acid Type B Receptors Regulating Cholecystokinin and Somatostatin Release from Rat Neocortex Synaptosomes

ANITA GEMIGNANI, PAOLO PAUDICE, GIAMBATTISTA BONANNO, and MAURIZIO RAITERI

Institute of Pharmacology and Pharmacognosy, University of Genoa, 16148 Genoa, Italy

Received March 7, 1994; Accepted June 13, 1994

SUMMARY

The γ-aminobutyric acid $(GABA)_B$ receptors modulating the depolarization-evoked release of somatostatin (SRIF) or cholecystokinin (CCK) from superfused rat cerebrocortical synaptosomes have been characterized pharmacologically. GABA inhibited the 15 mM KCl-evoked overflow of both SRIF and CCK; the EC_{50} values were 1.3 μM and 1.4 μM, respectively. The $GABA_B$ receptor agonist (−)-baclofen also diminished the release of SRIF (EC_{50} = 1.9 μM) and CCK (EC_{50} = 2.6 μM). The novel compound CGP 47656, a highly selective $GABA_B$ receptor ligand, inhibited the release of SRIF, with its affinity and efficacy being similar to those of GABA or (−)-baclofen; however, the compound was unable to affect CCK release even when tested at 300 μM. The $GABA_B$ receptor antagonist phaclofen prevented, with identical affinities, the effects of (−)-baclofen on SRIF (pK_b = 4.9) and CCK (pK_b = 4.8) release. The same was true for CGP 35348, another $GABA_B$ receptor antagonist, which blocked (−)-baclofen with a pK_b value of 6.1 at both the $GABA_B$ receptors regulating SRIF and CCK release. The effects of (−)-baclofen were also counteracted by the novel $GABA_B$ receptor antagonist CGP 52432. However, the affinity of the drug at the $GABA_B$ receptors modulating SRIF release (pK_b = 6.2) was about 30-fold lower than that at the receptors regulating CCK release (pK_b = 7.6). The data suggest that the $GABA_B$ receptors situated on nerve terminals releasing SRIF and CCK display pharmacological heterogeneity and may represent different subtypes of $GABA_B$ receptors.

Evidence is accumulating that $GABA_B$ receptors having different neuronal locations and functions in the central nervous system also display distinct pharmacological profiles. The existence and properties of multiple subtypes of $GABA_B$ receptors in the mammalian brain have been recently reviewed (1). A major function of $GABA_B$ receptors is to mediate inhibition of neurotransmitter release at the presynaptic level (for reviews, see Refs. 1–3). For this reason, comparison of presynaptic release-regulating $GABA_B$ receptors by using the newly available $GABA_B$ receptor ligands [see review by Bittiger *et al.* (4)] represents a rational approach to the identification of $GABA_B$ receptor subtypes.

In a previous work it was reported that GABA can inhibit the depolarization-evoked release of SRIF in rat cerebrocortex by activating $GABA_B$ receptors located on SRIF-releasing nerve terminals (5). More recently we have observed the K^+-evoked overflow of another neuropeptide, CCK, to be sensitive to $GABA_B$ receptor activation (see below). The identification of receptor subtypes is viewed as an important opening in the development of more selective drugs. In particular, the likely involvement of SRIF and CCK in important pathological conditions, including Alzheimer dementia (6–8) and anxiety (9, 10), justifies efforts aimed at selectively modulating their synaptic availability.

In this work the $GABA_B$ receptors regulating SRIF and CCK release have been compared by using two novel ligands (see Fig. 1) that are highly selective for brain $GABA_B$ receptors, i.e., CGP 47656[1] and CGP 52432 (11), in addition to the well established $GABA_B$ receptor antagonists phaclofen (12) and CGP 35348 (13). The two $GABA_B$ receptors tested are differentially sensitive to the novel compounds CGP 47656 and CGP 52432, suggesting pharmacological heterogeneity.

Experimental Procedures

Animals. Adult male Sprague-Dawley rats (200–250 g) were used. Animals were housed at constant temperature (22 ± 1°) and relative

This work was supported by grants from the Italian Ministero dell' Università e della Ricerca Scientifica e Tecnologica (40 and 60%) and from the Italian Consiglio Nazionale delle Ricerche.

[1] H. Bittiger (Ciba Geigy, Basel, Switzerland), personal communication.

ABBREVIATIONS: GABA, γ-aminobutyric acid; SRIF, somatostatin; CCK, cholecystokinin; CGP 47656, 3-aminopropyl(difluoromethyl)phosphinic acid; CGP 52432, [3-[[(3,4-dichlorophenyl)methyl]amino]propyl](diethoxymethyl)phosphinic acid; CGP 35348, 3-aminopropyl(diethoxymethyl)-phosphinic acid; 5-HT, 5-hydroxytryptamine.

humidity (50%) on a regular light-dark schedule (lights on from 7.00 a.m. to 7.00 p.m.). Food and water were freely available.

Preparation of synaptosomes. Rats were killed by decapitation, the brains were rapidly removed, and the cortices were dissected. Crude synaptosomes were prepared as described previously (14). The synaptosomal pellet was then resuspended in a physiological medium having the following composition (in mM): NaCl, 125; KCl, 3; $MgSO_4$, 1.2; $CaCl_2$, 1.2; NaH_2PO_4, 1.0; $NaHCO_3$, 22; glucose, 10; pH 7.2–7.4 (aerated with 95% O_2/5% CO_2 at 37°).

Release experiments. Identical aliquots of the synaptosomal suspensions were layered on microporous filters at the bottom of a set of parallel superfusion chambers maintained at 37° (15). Superfusion was then started at a rate of 0.5 ml/min with standard medium, supplemented with 0.1% bovine serum albumin or gelatin in the experiments on SRIF or CCK release, respectively, and aerated with 95% O_2/5% CO_2. After 36 min, to equilibrate the system, fractions were collected according to the following scheme: two 3-min samples (basal release) before and after one 6-min sample (evoked release). A 90-sec period of depolarization (15 mM KCl) was applied after the first fraction had been collected. GABA, (−)-baclofen, or CGP 47656 (when tested as an agonist) was added to the superfusion medium concomitantly with the depolarizing stimulus. Phaclofen, CGP 35348, CGP 52432, or CGP 47656 (when used as an antagonist) was added 8 min before (−)-baclofen. Superfusate fractions were collected into vials containing acetic acid (final concentration, 1 M), and aliquots were boiled to ensure peptidase inhibition. Superfusate fractions were freeze dried before assay.

In one set of experiments, cerebrocortex synaptosomes were labeled with 0.04 μM [^{3}H]GABA and depolarized by superfusion with 9 mM KCl to test the activity of CGP 47656 at the GABA autoreceptors (16). Fractions collected and superfused synaptosomes were counted for radioactivity.

Radioimmunoassay. The freeze-dried samples were reconstituted in phosphate buffer (50 mM; pH 7.4) and radioimmunoassayed. The assay sensitivity reached with the CCK antiserum used (Ab 2717) (17) amounted to 1 pg/tube. The SRIF antiserum used (Rb 143) was characterized in detail previously; the assay sensitivity was 1 pg/tube (18).

Calculations. The amounts of CCK or SRIF released in each fraction were expressed as picograms/milligram of protein. [^{3}H]GABA release was evaluated as fractional rate. The depolarization-evoked overflow was estimated by subtracting the peptide (or [^{3}H]GABA) content of basal release from the content in the 6-min fraction collected during and after the depolarization period.

Drug effects were evaluated as the ratio of the depolarization-evoked overflow calculated in the presence of drugs to that calculated under control conditions. Appropriate controls with antagonists were always run in parallel. The effects of the GABA$_B$ receptor antagonists were expressed as percentages of the inhibitory effect of (−)-baclofen.

EC_{50} and IC_{50} values were determined from the experimental data by using a function-fitting routine provided by the software SigmaPlot, Windows version 1.01. K_b values for antagonists were calculated according to a modification of the equation of Cheng and Prusoff (19) proposed by Lazareno and Birdsall (20). The statistical significance of the experiments shown in Fig. 4 was assayed by Dunnett's test.

Chemicals. [^{3}H]GABA (specific activity, 85.2 Ci/mmol), ^{125}I-CCK-8, and ^{125}I-(Tyr$_{11}$)SRIF-14 were obtained from Amersham Radiochemical Centre (Buckinghamshire, UK). CCK-8 and SRIF-14 (SRIF-28$_{14-28}$) were from NOVA Biochem (Läufelfingen, Switzerland). Phaclofen was purchased from Tocris Neuramin (Bristol, UK). CGP 35348, CGP 52432, CGP 47656, and (−)-baclofen were kindly donated by Ciba Geigy (Basel, Switzerland).

The pharmacological profile of the GABA$_B$ receptor ligand CGP 35348 has been reported previously (13). For the more recent compounds CGP 47656 and CGP 52432, the following radioligand binding data[2] show that both molecules are highly selective GABA$_B$ receptor ligands. For CGP 47656, the IC_{50} at GABA$_B$ receptors was 77 nM, the IC_{50} at GABA$_A$ sites was 135 μM, and there was no interaction with α_1-, α_2-, or β-adrenergic, muscarinic cholinergic, 5-HT$_1$, 5-HT$_2$, or histamine$_1$ sites at up to 1 mM. For CGP 52432, the IC_{50} at GABA$_B$ receptors was 55 nM and there was no interaction (at up to 10 μM) with GABA$_A$, α_1-adrenergic, 5-HT$_1$, 5-HT$_2$, or 5-HT$_3$ sites.

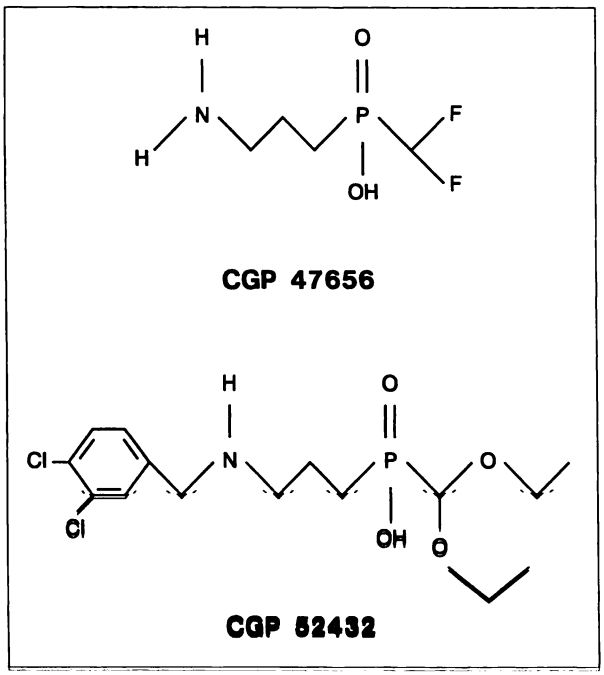

Fig. 1. Chemical structures of the compounds CGP 47656 and CGP 52432.

Results

GABA, (−)-baclofen, and CGP 47656 inhibited in a concentration-dependent manner the K^+ (15 mM)-evoked release of SRIF from cerebrocortical synaptosomes (Fig. 2, *left*). The three compounds displayed almost identical affinities and efficacies (EC_{50} of approximately 1–2 μM; maximum effect of almost 50% inhibition).

GABA and (−)-baclofen also inhibited the K^+ (15 mM)-evoked release of CCK. The respective concentration-inhibition curves were practically superimposable (Fig. 2, *right*). The two agonists exhibited EC_{50} values and maximal effects very similar to those obtained when the two compounds were tested on SRIF release (Fig. 2; Table 1). In contrast, the compound CGP 47656 did not affect CCK release, even when tested at a concentration as high as 300 μM (Fig. 2, *right*).

The effects of (−)-baclofen (10 μM) on the two neuropeptide release systems were then tested against increasing concentrations of GABA$_B$ receptor-selective antagonists. Phaclofen and CGP 35348 shared the same potency towards the (−)-baclofen-induced inhibition of the release of SRIF (Fig. 3, *left*) and CCK (Fig. 3, *right*). As summarized in Table 1, the pK_b values of phaclofen were 4.8 (CCK release) and 4.9 (SRIF release); the pK_b values of CGP 35348 were 6.1 (CCK release) and 6.1 (SRIF release).

When the novel GABA$_B$ receptor antagonist CGP 52432 was tested, the inhibitions by (−)-baclofen of the release of the two peptides were affected differentially (Fig. 3); CGP 52432 was about 30 times less potent at the GABA$_B$ receptors inhibiting

[2] H. Bittiger, personal communication.

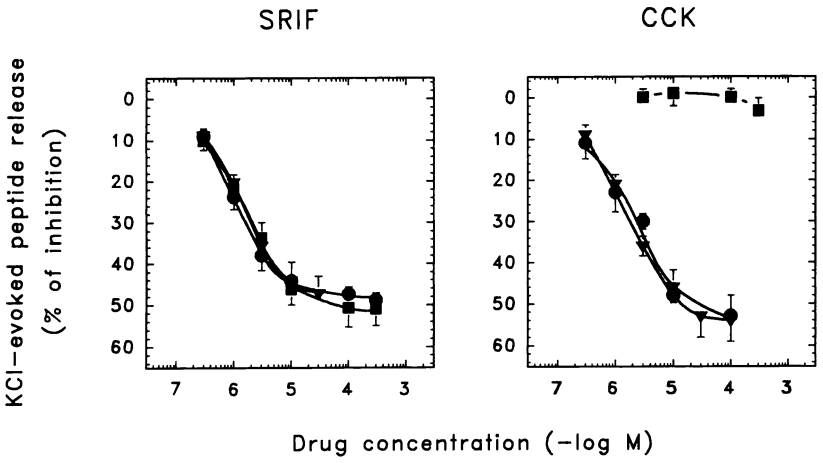

Fig. 2. Effects of GABA (▼), (−)-baclofen (●), and CGP 47656 (■) on the K^+ (15 mM)-evoked release of SRIF and CCK from rat brain cortex synaptosomes. Agonists were added to the superfusion medium concomitantly with the depolarizing stimulus. The spontaneous outflow of SRIF and CCK was 6.2 ± 1.1 pg/mg of protein/min (nine experiments) and 4.0 ± 0.5 pg/mg of protein/min (27 experiments), respectively. The depolarization-evoked overflow amounted to 95.3 ± 13.0 pg/mg of protein (SRIF) and 32.0 ± 3.8 pg/mg of protein (CCK). Under the experimental conditions used, the 15 mM K^+-stimulated overflow of the two peptides was found to be almost completely calcium dependent (17, 21). See Experimental Procedures for other technical details. The data presented are means ± standard errors of four to 18 experiments in triplicate. The effect of GABA on SRIF release was taken from the report of Bonanno and Raiteri (24).

TABLE 1

EC_{50} and pK_b values for agonists and antagonists at the $GABA_B$ receptors mediating inhibition of SRIF or CCK release

Antagonists were tested at varying concentrations against 10 μM (−)-baclofen. pK_b values were calculated from the IC_{50} values according to the method of Lazareno and Birdsall (20). EC_{50} and IC_{50} values were deduced from the fitted curves in Figs. 2 and 3. See Experimental Procedures for additional technical details.

	SRIF		CCK	
	EC_{50}	pK_b	EC_{50}	pK_b
	μM		μM	
GABA	1.3[a]		1.4	
(−)-Baclofen	1.9		2.6	
CGP 47656	1.3		>300	
Phaclofen		4.9[b]		4.8
CGP 35348		6.1[b]		6.1
CGP 52432		6.2[c]		7.6

[a] Taken from the report of Bonanno and Raiteri (24).
[b] Recalculated from the data of Bonanno and Raiteri (24).
[c] Recalculated from the data of Lanza *et al.* (25).

SRIF release (pK_b = 6.2) than at the receptors inhibiting CCK release (pK_b = 7.6).

Phaclofen (100 μM), CGP 35348 (30 μM), and CGP 52432 (30 μM) also significantly antagonized the inhibition by 10 μM CGP 47656 of the K^+-evoked release of SRIF (Fig. 4). Moreover, the compound CGP 47656, which behaves as an agonist at the $GABA_B$ receptors regulating SRIF release but not at those regulating CCK release, did not antagonize the inhibition by (−)-baclofen of the release of CCK (Fig. 3, *right*). When tested on the K^+-evoked overflow of [^{3}H]GABA, CGP 47656 was ineffective as an autoreceptor agonist at up to 300 μM (data not shown); the drug could, however, antagonize (pK_b = 6.1) the inhibitory effect of 10 μM (−)-baclofen.

Discussion

The results of the present investigation confirm previous data showing that the depolarization-evoked overflow of SRIF (5) from rat neocortex synaptosomes can be inhibited by GABA acting at receptors of the $GABA_B$ type. The experiments on CCK release indicate also that the release of this peptide can be regulated through the activation of inhibitory receptors belonging to the $GABA_B$ type. The use of isolated nerve terminals and the characteristics of the technique used to study release (a thin layer of synaptosomes up-down superfused, in which indirect effects are minimized and endogenous compounds released are rapidly removed from the receptor biophase, thus permitting evaluation of true affinities) (15) allow us to conclude that the $GABA_B$ receptors mediating inhibition of SRIF and CCK release are probably situated on the peptide-releasing terminals. As to the peptide species released during K^+ depolarization, chromatographic analysis carried out on superfusate samples showed that the immunoreactive substances released essentially consist of SRIF-14 (21) and CCK-8 sulfate (22), respectively.

The $GABA_B$ receptors regulating SRIF and CCK release exhibited identical sensitivities to the natural agonist GABA

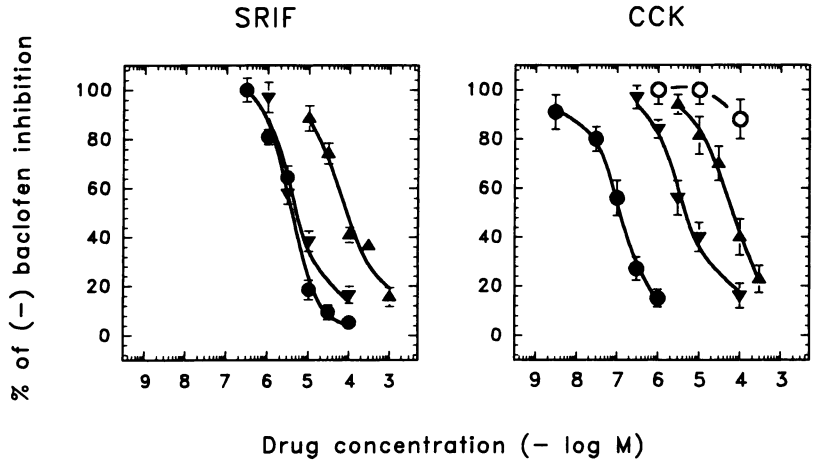

Fig. 3. Antagonism by CGP 35348 (▼), CGP 52432 (●), phaclofen (▲), or CGP 47656 (○) of the 10 μM (−)-baclofen-induced inhibition of SRIF or CCK release evoked by 15 mM K^+ depolarization in rat brain cortex synaptosomes. CGP 47656 was tested as an antagonist only for CCK release. Synaptosomes were exposed to (−)-baclofen concomitantly with high K^+ stimulus and to antagonists 8 min before high K^+ stimulus. See Experimental Procedures for additional technical details. Data are means ± standard errors of three to eight experiments in triplicate. The effects of phaclofen and CGP 35348 and of CGP 52432 on SRIF release are taken from the reports of Bonanno and Raiteri (24) and Lanza *et al.* (25), respectively.

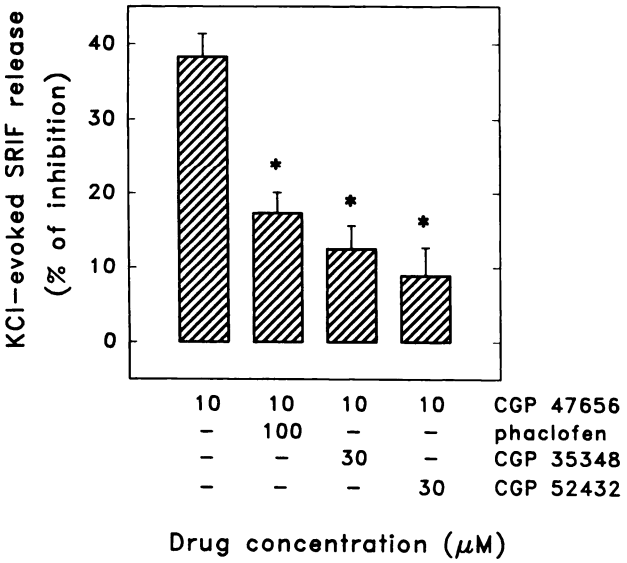

Fig. 4. Antagonism by phaclofen, CGP 35348, and CGP 52432 of the CGP 47656-induced inhibition of SRIF release evoked by 15 mM KCl in rat brain cortex synaptosomes. CGP 47656 was added concomitantly with the depolarizing stimulus, and antagonists were added 8 min before the stimulus. See Experimental Procedures for other technical details. Data are means ± standard errors of three or four experiments in triplicate. *, $p < 0.05$ (Dunnett's test).

TABLE 2

pK_b values for antagonists at the GABA$_B$ receptor subtypes present in rat cerebral cortex

Increasing concentrations of antagonists were tested against 10 μM (−)-baclofen. pK_b values were calculated from the IC$_{50}$ values according to the method of Lazareno and Birdsall (20).

	pK_b			
	GABA$_{B1\alpha}$	GABA$_{B1\beta}$	GABA$_{B1\gamma}$	GABA$_{B1\delta}$
Phaclofen	5.1	Inactive	4.9	4.8
CGP 35348	Inactive	6.2	6.1	6.1
CGP 52432	7.9	5.7	6.2	7.6
CGP 47656	6.1	ND[a]	Full agonist	Inactive

[a] ND, not determined.

and to the classic GABA$_B$ receptor agonist (−)-baclofen (Fig. 2; Table 1). However, CGP 47656, a novel compound showing a binding profile highly selective for brain GABA$_B$ receptors, behaved differently at the two receptors. As shown in Fig. 2, CGP 47656 was a full agonist at the GABA$_B$ receptor regulating SRIF release, with its affinity (EC$_{50}$ = 1.3 μM) being very similar to those of GABA (1.3 μM) and (−)-baclofen (1.9 μM). In contrast, the drug did not affect CCK release at up to 300 μM. One may object that agonists may not be appropriate tools to establish receptor heterogeneity for a number of reasons, including the involvement of transduction mechanisms and the presence of spare receptors. On the other hand, CGP 47656 was also unable to prevent the inhibition of the CCK release caused by (−)-baclofen, suggesting that the compound cannot be easily recognized by the GABA$_B$ receptors regulating CCK release. Interestingly, CGP 47656 did antagonize (pK_b = 6.1) (Table 2) the GABA$_B$ autoreceptors regulating GABA release from cortical synaptosomes. Thus, it is tempting to conclude that the receptors situated on CCK-releasing terminals in neocortex, certainly representing a small percentage of the total brain GABA$_B$ receptors, differ from the majority of these receptors, to which CGP 47656 was shown to bind with high affinity (IC$_{50}$ = 77 nM).

In particular, the GABA$_B$ receptors present on CCK-releasing terminals may differ from the GABA$_B$ receptors situated on SRIF-releasing terminals. The results obtained with the novel GABA$_B$ receptor antagonist CGP 52432 and shown in Fig. 3 support this view. The affinity of the drug for the GABA$_B$ receptor situated on CCK nerve endings is about 30-fold higher than the affinity for the receptor present on SRIF-releasing terminals. Antagonists are considered appropriate tools for investigating pharmacological receptor heterogeneity (23). Moreover, a difference of >10-fold between antagonist affinities is often considered compatible with the existence of pharmacological receptor subtypes. Additional support for the idea that SRIF- and CCK-releasing terminals possess distinct GABA$_B$ receptors comes from the observation (see Table 1) that CGP 35348 and CGP 52432 show identical affinities for the receptor regulating SRIF release, whereas the affinity of CGP 52432 is higher (about 30-fold) than that of CGP 35348 at the receptor regulating CCK release. To conclude, the receptors situated on the SRIF- and CCK-releasing nerve endings are likely to represent pharmacologically distinct subtypes of GABA$_B$ receptors.

It was previously proposed that, in the rat cerebrocortex, three subtypes of (−)-baclofen-sensitive GABA$_B$ receptors exist (see Table 2), 1) a phaclofen-sensitive, CGP 35348-insensitive receptor situated on GABAergic terminals and mediating inhibition of GABA release, 2) a phaclofen-insensitive, CGP 35348-sensitive receptor situated on glutamatergic terminals and mediating inhibition of glutamic acid release, and 3) a phaclofen-sensitive, CGP 35348-sensitive receptor located on SRIF terminals and mediating inhibition of SRIF release. These receptors were termed GABA$_{B1\alpha}$, GABA$_{B1\beta}$, and GABA$_{B1\gamma}$, respectively (24). As shown in Table 2, the experiments performed with the novel compounds CGP 52432 and CGP 47656 provide additional evidence for the aforementioned idea of three distinct (−)-baclofen-sensitive GABA$_B$ receptors. The impressive high affinity of CGP 52432 for the autoreceptor (25) confirms that the GABA$_{B1\alpha}$ receptor differs pharmacologically from both the GABA$_{B1\beta}$ and GABA$_{B1\gamma}$ receptors. The difference between GABA$_{B1\alpha}$ and GABA$_{B1\gamma}$ receptors is strengthened by the data for CGP 47656, which is an antagonist at the former but an agonist at the latter. Moreover, the present results seem to add further complexity to the GABA$_B$ receptor family by suggesting the existence on CCK-releasing terminals of yet another subtype of GABA$_B$ receptors sensitive to (−)-baclofen, here indicated as the GABA$_{B1}\delta$ receptor (Table 2). It may be worthwhile to recall that a subtype of the GABA$_B$ receptor that is insensitive to (−)-baclofen but sensitive to selective GABA$_B$ receptor agonists and antagonists (termed GABA$_{B2}$) was found to exist in the rat spinal cord, where it appears to function as an autoreceptor (1, 26).

In conclusion, GABA$_B$ receptors can mediate inhibition of SRIF and CCK release. These receptors may represent distinct subtypes of the GABA$_B$ receptor, in turn being different from previously characterized subtypes. Thus, on the basis of functional pharmacological evidence, receptors of the GABA$_B$ type seem to constitute a highly heterogeneous family. It is hoped that such heterogeneity will foster molecular biology studies aimed at the long awaited but still unsuccessful cloning of

$GABA_B$ receptors, thus providing the necessary structural basis for the observed pharmacological multiplicity.

Acknowledgments

The authors wish to thank Dr. Helmut Bittiger (Ciba Geigy, Basel, Switzerland) for kindly providing the binding profiles of CGP 47656 and CGP 52432. The skillful secretarial assistance of Mrs. Maura Agate is also acknowledged.

References

1. Bonanno, G., and M. Raiteri. Multiple $GABA_B$ receptors. *Trends Pharmacol. Sci.* **14**:259–261 (1993).
2. Bowery, N. G. $GABA_B$ receptors and their significance in mammalian pharmacology. *Trends Pharmacol. Sci.* **10**:401–407 (1989).
3. Bowery, N. G. $GABA_B$ receptor pharmacology. *Annu. Rev. Pharmacol. Toxicol.* **33**:109–147 (1993).
4. Bittiger, H., W. Fröstl, S. J. Mickel, and H.-R. Olpe. $GABA_B$ receptor antagonists: from synthesis to therapeutic applications. *Trends Pharmacol. Sci.* **14**:391–394 (1993).
5. Bonanno, G., A. Gemignani, E. Fedele, G. Fontana, and M. Raiteri. γ-Aminobutyric $acid_B$ ($GABA_B$) receptors mediate inhibition of somatostatin release from cerebrocortex nerve terminals. *J. Pharmacol. Exp. Ther.* **259**:1153–1159 (1991).
6. McDonald, W. M., and C. B. Nemeroff. Neurotransmitters and neuropeptides in Alzheimer's disease. *Psychiatr. Clin. North. Am.* **14**:421–442 (1991).
7. Bissette, G., and B. Myers. Somatostatin in Alzheimer's disease and depression. *Life Sci.* **51**:1389–1410 (1992).
8. Krantic, S., Y. Robitaille, and R. Quirion. Deficits in the somatostatin SS1 receptor subtype in frontal and temporal cortices in Alzheimer's disease. *Brain Res.* **573**:299–304 (1992).
9. Ravard, S., and C. T. Dourish. Cholecystokinin and anxiety. *Trends Pharmacol. Sci.* **11**:271–273 (1990).
10. Woodruff, G. N., and J. Hughes. Cholecystokinin antagonists. *Annu. Rev. Pharmacol. Toxicol.* **31**:469–501 (1991).
11. Fröstl, W., S. J. Mickel, G. Von Sprecher, H. Bittiger, and H.-R. Olpe. Chemistry of new $GABA_B$ antagonists. *Pharmacol. Commun.* **2**:52–56 (1992).
12. Kerr, D. I. B., J. Ong, R. H. Prager, B. D. Gynther, and D. R. Curtis. Phaclofen: a peripheral and central baclofen antagonist. *Brain Res.* **405**:150–154 (1987).
13. Olpe, H.-R., G. Karlsson, M. F. Pozza, F. Brugger, M. Steinmann, H. Van Riezen, G. Fagg, R. G. Hall, W. Fröstl, and H. Bittiger. CGP 35348: a centrally active blocker of $GABA_B$ receptors. *Eur. J. Pharmacol.* **187**:27–38 (1990).
14. Raiteri, M., G. Bonanno, M. Marchi, and G. Maura. Is there a functional linkage between neurotransmitter uptake mechanisms and presynaptic receptors? *J. Pharmacol. Exp. Ther.* **231**:671–677 (1984).
15. Raiteri, M., F. Angelini, and G. Levi. A simple apparatus for studying the release of neurotransmitters from synaptosomes. *Eur. J. Pharmacol.* **25**:411–414 (1974).
16. Pittaluga, A., D. Asaro, G. Pellegrini, and M. Raiteri. Studies on ^{3}H-GABA and endogenous GABA release in rat cerebral cortex suggest the presence of autoreceptors of the $GABA_B$ type. *Eur. J. Pharmacol.* **144**:45–52 (1987).
17. Paudice, P., and M. Raiteri. Cholecystokinin release mediated by 5-HT_3 receptors in rat cerebral cortex and nucleus accumbens. *Br. J. Pharmacol.* **103**:1790–1794 (1991).
18. Dawbarn, D., M. E. Dequidt, and P. C. Emson. Survival of basal ganglia neuropeptide Y/somatostatin neurons in Huntington's disease. *Brain Res.* **340**:251–260 (1985).
19. Cheng, Y. C., and W. H. Prusoff. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).
20. Lazareno, S., and N. J. M. Birdsall. Estimation of antagonist K_b from inhibition curves in functional experiments: alternatives to the Cheng-Prusoff equation. *Trends Pharmacol. Sci.* **14**:237–239 (1993).
21. Bonanno, G., B. Parodi, S. Cafaggi, and M. Raiteri. Somatostatin release from rat cerebral cortex synaptosomes. *J. Neurochem.* **57**:1258–1264 (1991).
22. Verhage, M., W. E. J. M. Ghijsen, D. G. Nicholls, and V. M. Wiegant. Characterization of the release of cholecystokinin-8 from isolated nerve terminals and comparison with exocytosis of classical transmitters. *J. Neurochem.* **56**:1394–1400 (1991).
23. Hall, J. M., M. P. Caulfield, S. P. Watson, and S. Guard. Receptor subtypes or species homologues: relevance to drug discovery. *Trends Pharmacol. Sci.* **14**:376–383 (1993).
24. Bonanno, G., and M. Raiteri. Functional evidence for multiple $GABA_B$ receptor subtypes in the rat cerebral cortex. *J. Pharmacol. Exp. Ther.* **262**:114–118 (1992).
25. Lanza, M., A. Fassio, A. Gemignani, G. Bonanno, and M. Raiteri. CGP 52432: a novel potent and selective $GABA_B$ autoreceptor antagonist in rat cerebral cortex. *Eur. J. Pharmacol.* **237**:191–195 (1993).
26. Bonanno, G., and M. Raiteri. γ-Aminobutyric acid (GABA) autoreceptors in rat cerebral cortex and spinal cord represent pharmacologically distinct subtypes of the $GABA_B$ receptor. *J. Pharmacol. Exp. Ther.* **265**:765–770 (1993).

Send reprint requests to: Maurizio Raiteri, Istituto di Farmacologia e Farmacognosia, Viale Cembrano 4, 16148 Genova, Italy.