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Lack of coupling between GABA release and GABA synthesis in the rat brain via $GABA_B$ autoreceptors

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Summary. GABA is synthesized within GABA terminals through a highly compartmentalized process in which glial-derived glutamine is a major precursor and its release is modulated by $GABA_B$ autoreceptors. The aim of this work was to ascertain whether or not GABA synthesis and release are coupled in the rat brain through a $GABA_B$ autoreceptor-mediated modulation. It was found that (−)baclofen (30 μM) reduces the K^+ stimulated release of [^{3}H]GABA in synaptosomes and prisms (10 μM) from cerebral cortex, while at the same concentrations (−)baclofen failed to modify the synthesis of [^{3}H]GABA from [^{3}H]glutamine in cortical and hypothalamic slices, prisms and in cortical synaptosomes. In this latter preparation, identical results were observed when (−)baclofen was added to Krebs-Tris media, containing 5 or 15 mM K^+ concentration. In agreement with these latter results, glutamic acid decarboxylase (GAD) activity from cortical and hypothalamic prisms was not affected by 1–100 μM (−)baclofen. Similar results on GABA synthesis were also observed when 1–100 μM 3-aminopropil(methyl)-phosphinic acid or GABA was used instead of (−)baclofen to stimulate $GABA_B$ autoreceptors. [^{3}H]GABA release, [^{3}H]GABA synthesis from [^{3}H]glutamine and GAD activity were also insensitive to the action of the $GABA_B$ antagonist CGP 52432 (10–100 μM). Likewise, muscimol (0.3–100 μM) did not affect GABA synthesis. Our results indicate that unlike GABA release, GABA synthesis is not modulated by $GABA_B$ autoreceptors.

Keywords: Amino acids – GABA-synthesis – GABA-release – GABA – (−)Baclofen – $GABA_B$ autoreceptors – CGP 52432 – Rat brain

Introduction

Neurotransmitter release, a process of paramount importance in synaptic function, is a tightly modulated mechanism. Considerable evidence has

accumulated during the last years indicating that presynapic autoreceptors (Carlsson, 1975) play a major modulatory influence on the release of a number of neurotransmitters, for example dopamine (DA; Romo et al., 1986; Elsworth and Roth, 1996), serotonin (Engel et al., 1986; Limberger et al., 1989; Rollema et al., 1996), noradrenaline (Kirpehar and Puig, 1971) acetylcholine (Nordström and Bartfai, 1980; Baghdoyan et al., 1998), glutamate (Lovinger, 1991; Herrero et al., 1992; Cochilla and Alford, 1998) and GABA (Raiteri et al., 1989; Waldmeier and Baumman, 1990; Langer, 1997). Moreover within the aminergic neuronal systems, the same type of autoreceptor modulates neurotransmitter release and neurotransmitter synthesis (Carlsson, 1975; Meller et al., 1990; Westernik et al., 1990; Elsworth and Roth, 1996; Gainetdinov et al., 1996); this indicates the existence of a coupling between neurotransmitter release and synthesis. In support of this comodulation, it has been shown that the activation of DA D_2 type autoreceptors decreases tyrosine hydroxylase (TH) activity and DA release. TH inhibition seems to occur as a result of a modification of its phosphorylation state (Wolf and Roth, 1990; Goldstein, 1995) and it has been shown to be responsible for a similar reduction (50%) in the quantal size of the DA released (Pothos et al., 1998).

In regard to the GABA system, several findings suggest that a similar comodulation may exist. Thus, metabotropic $GABA_B$ autoreceptors regulate GABA release (Langer, 1977; Raiteri et al., 1989; Waldmeier and Baumman, 1990; see also Bowery, 1993 for a review on $GABA_B$ receptors) and glutamic acid decarboxylase (GAD), the enzyme responsible for the rate-limiting step in the synthesis of GABA, is also modulated through a putative phosphorylation-dephosphorylation cycle (Bao et al., 1995). Furthermore, it has been reported that the activation of $GABA_B$ receptors by the GABA agonist (−)baclofen (Bowery, 1993) inhibits the calcium and depolarization dependent-TH activity in the striatum of the rat (Arias-Montaño et al., 1991). The aim of this work is to study whether GABA release and GABA synthesis are comodulated by metabotropic $GABA_B$ autoreceptors. The participation of $GABA_A$ autoreceptors on this hypothetical comodulation is also explored.

Material and methods

Animals and brain preparations

Male Wistar rats (180–200 g body weight) were used. The animals were kept on a normal 12:12 h light-dark cycle and had food and water *ad libitum*. Animals were killed by decapitation and their brains were quickly excised from the skull and submerged into a cold saline solution. The hypothalamus and the frontoparietal cerebral cortex were manually dissected out from coronal slices with the aid of two parallel razor blades positioned in the hypothalamus between the preoptic and the mammilary bodies, and in the cerebral cortex between the rostral border of the optic chiasma and an imaginary line drawn 3 mm ahead. Hypothalamic slices (400 μm) were obtained with a Mc Ilwain tissue chopper by slicing the hypothalamus perpendicularly to its ventral side. Cortical and hypothalamic prisms were obtained by slicing (300 μm) each region in two perpendicular directions. Cortical synaptosomes were prepared by the method of Löscher et al. (1985) and suspended in a standard Krebs-Tris medium of the following composition: 124 mM

NaCl, 5 mM KCl, 1.25 mM KH_2PO_4, 1.2 mM $MgSO_4$-$7H_2O$, 35 mM Trizma base, 10 mM glucose and 0.75 mM $CaCl_2$, pH 7.2 gassed with 100% O_2.

[^{3}H]GABA release

[^{3}H]GABA release was measured in the presence and in the absence of (−)baclofen essentially as described by Pérez de la Mora et al. (1993). Cortical prisms were equilibrated for 30 min at 37°C in a Krebs-Tris medium. After this time [^{3}H]GABA (45 Ci/mmol specific activity) was added to a final concentration of 0.4 μM and the incubation was continued for 15 min to allow sufficient neuronal uptake of [^{3}H]GABA. Hundred μM β-alanine was present during this period to prevent the [^{3}H]GABA uptake by the glia (Schon and Kelly, 1974; Raiteri et al., 1989). At the end of this loading period, the prisms were transfered to superfusion chambers with a volume of 0.25 ml and superfused at a flow rate of 1.5 ml/min with standard Krebs-Tris medium supplemented with 10 μM aminooxyacetic acid. After 40 min new Krebs-Tris medium that contained (−)baclofen or CGP 52432 was superfused into the experimental chambers. Ten min afterwards the prisms in control and in experimental chambers were stimulated with 15 mM or 30 mM K^+ (CGP 52432 experiments) for 10 min and the superfusion was continued as before for 5 min. To prevent [^{3}H]GABA breakdown 10 μM aminooxyacetic acid was present from the [^{3}H]GABA loading till the end of the superfusion. When the KCl concentration was increased in the media, the isotonicity was maintained by reducing the NaCl concentration. (−)Baclofen or CGP 52432 was present in the superfusion media of the experimental chambers from its introduction 10 min before the stimulation until the end of the experiment. Nipecotic acid was present during the superfusion to prevent [^{3}H]GABA uptake into GABA terminals (Krogsgaard-Larsen and Johnston, 1975). Equilibration, loading and superfusion were always carried out at 37°C. Fractions were collected every minute after 15–25 min of superfusion; at the end of the experiment the prisms were digested in 0.5 ml 1% sodium dodecyl sulfate. The radioactivity in both prisms and fractions was counted by scintillation spectrometry in vials containing 5 ml Tritosol.

To measure release from synaptosomes 300 μg synaptosomal protein was incubated in a Krebs-Tris medium similar to that used for the incubation of brain prisms. At the end of the incubation period, aliquots of the synaptosomal suspension were distributed on Millipore filters (0.65 μm) and superfused as described originally by Raiteri et al. (1974) with some modifications. In particular, the filters containing the trapped synaptosomes were cut into pieces so that they could be introduced into 0.25 ml superfusion chambers. Superfusion was carried out as for the experiments of [^{3}H]GABA release from prisms.

We have already shown (Pérez de la Mora et al., 1993) that under the above conditions, 90% of the radioactivity released by the K^+ stimulation comigrated with authentic GABA and that 80% of the radioactivity stored within the prisms was [^{3}H]GABA. Thus, we will refer to the radioactivity released during the superfusion and the radioactivity present in the preparation used, at the onset of K^+ stimulation as [^{3}H]GABA. [^{3}H]GABA released under the above conditions has been shown to be a least 70% Ca^{2+} dependent (Pérez de la Mora et al., 1993). The efflux of [^{3}H]GABA was expressed in percent of the total [^{3}H]GABA existing in the brain preparation at the onset of the K^+-stimulation.

GABA synthesis

Overall [^{3}H]GABA synthesis was determined from the incorporation of radioactivity from [^{3}H]glutamine into [^{3}H]GABA. GAD activity within brain prisms was also evaluated under control and experimental conditions. The methodology used for both procedures has been published in detail elsewhere (Pérez de la Mora et al., 1999). To measure the overall [^{3}H]GABA synthesis from [^{3}H]glutamine, prisms (0.3–0.7 mg protein) were equilibrated at 37°C for 40 min in 450 μl standard Krebs-Tris medium in

chambers filled with latex stoppers which allow the introduction of syringe needles to inject substances or to change the atmosphere of the chamber. Five μCi [^{3}H]glutamine (30 Ci/mmol) diluted in 50 μl standard Krebs-Tris medium were then injected to reach a 0.33 μM final concentration and the incorporation of radioactivity from [^{3}H]glutamine into [^{3}H]GABA was allowed to proceed for 10 min. [^{3}H]GABA synthesis was stopped by transferring the incubation chambers into an ice-salt-cooled water bath set at 0°C and by aspirating the radioactive medium. The prisms were washed with 3 ml portions of ice cold non-radioactive medium, followed by sonication in 1.5 ml of 80% ice-cold ethanol. After centrifugation (3,000 rev/min; 10 min), the supernatants were extracted with 5.0 ml chloroform and the radioactive amino acids were recovered from the water phase formed after a second centrifugation (3,000 rev/min; 10 min). A 100 μl aliquot from the water phase was freeze-dried and used for analysis. Blanks were made by cooling the incubation mixture just before the addition of [^{3}H]glutamine. Control experiments showed that the incorporation of radioactivity from [^{3}H]glutamine in [^{3}H]GABA was linear for at least 20 min. [^{3}H]GABA, was analyzed by high performance liquid chromatography (HPLC) using a Beckman (System Gold) chromatograph. The procedure involved reversed phase chromatography carried out under gradient conditions in a Ultrasphere column (ODS-DAB C18; 4.6 × 250 mm; Beckman Instruments) after precolumn derivatization. Dabsyl chloride(4-dimethylaminoazobenzene-4′-sulfonyl chloride) derivatization was performed using a Beckman Instruments kit. The mobile phase used to separate [^{3}H]GABA was formed by mixing phase A (0.01 M sodium citrate pH 6.5 in 4% dimethyl formamide (DMF)) and phase B (70% acetonitrile in 4% DMF) in a gradient in which the concentrations of phase A changed from 71% at 0 time to 41% in 12 min and then to 31% in 3 min. The column was washed with phase B only (4 min) and re-equilibrated to 71% phase A. The flow rate was, 1.4 ml/min. Under the above conditions [^{3}H]GABA appear in the chromatogram separated from all other amino acids as a sharp peak. The detection limit was 5 pmol GABA in the detector. The radioactivity in GABA was measured by scintillation spectrometry after addition of Tritosol in calibrated effluents of the column after their HPLC separation. The effects of the different treatments were evaluated from changes observed in the respective specific activities (dpm/pmol GABA). Control experiments showed that under the labeling conditions described 10 mM 3-mercaptopropionic acid, a well known GAD inhibitor (Lamar, 1970) decreased the incorporation of radioactivity from [^{3}H]glutamine into [^{3}H]GABA by 70% in cortical slices.

Glutamic acid decarboxylase (GAD) activity was measured radioisotopically in slices essentially as described by Pérez de la Mora et al. (1992), but using larger tubes to contain both the prisms and a small tube filled with 100 μl benzethonium chloride to trap the $^{14}CO_2$ evolved from L-[1^{14}C] glutamic acid during the reaction. The prisms (9–15 mg protein) were suspended and equilibrated at 37°C for 30 min in a standard Krebs-Tris medium. Drugs dissolved in the same medium were added to the desired concentrations. Five minutes later the reaction was initiated by the addition of DL[^{14}C]glutamic acid (5.46 Ci/mol; L form) to give a 3.6 μM concentration in 500 μl final volume; the reaction was stopped 20 min later. No pyridoxal phosphate was added to the incubation medium, since control experiments showed that under the conditions of the assay pyridoxal phosphate does not stimulate GAD activity. Time-course experiments showed that GAD activity is linear for at least 45 min; and other experiments showed that GAD activity is indeed measured within the slices.

Protein measurement

Protein was measured by the method of Lowry et al. (1951).

Statistics

Parametric procedures were used. For the release experiments, the effects of (−)baclofen on the K^+-stimulated release of [^{3}H]GABA were evaluated by comparing both the height,

and the area under the curve for the K^+-stimulated peak of experimental vs control superfusions. Student's "t" test was used to evaluate statistical significance. GABA synthesis experiments were evaluated by one-way, ANOVA analysis followed, when needed, by Dunnet's post-hoc test.

Material

Since in commercial [^{3}H]glutamine a [^{3}H]pyroglutamic-like compound is formed, a fresh lot of [3,4-^{3}H]glutamine (NEN, Dupont; Boston Ma, USA) was used for the [^{3}H]GABA synthesis experiments. [1-^{14}C] DL-glutamic acid was also from NEN. CGP 52432 ([3-[[(3,4-dichlorophenyl)methyl]amino]propyl](diethoymethyl)phosphinic acid) was a generous gift from Novartis Pharma AG, Basel, Switzerland. R-(+)baclofen hydrochloride (equivalent to (−)baclofen, free base), and 3-amino-propyl(methyl)phosphinic acid (3-APMPA) were purchased from Research Biochemicals International (RBI; Natick Ma, USA). Muscimol was obtained from Sigma Chemical Co. (St. Louis Misouri, USA) Tritosol was prepared according to Fricke (1975). All other chemicals were obtained from local sources and were of the purest grade available.

Results

Effects of $GABA_B$ receptor agonists on [^{3}H]GABA release

In agreement with previous work (Langer, 1977; Waldmeier and Baumman, 1970; Raiteri et al., 1989), the $GABA_B$ receptor agonist (−)baclofen (10μM) did not affect the basal [^{3}H]GABA release in cortical prisms, instead it diminished, in a statistical significant way, its K^+-stimulated release (Fig. 1A). A similar result was also observed when synaptosomes from the frontoparietal cerebral cortex were superfused with 30μM (−)baclofen (Fig. 1B).

Effects of GABA receptor ligands on tissue GABA levels

(−)Baclofen, 3-APMPA, CGP52432 and muscimol at the concentrations used in this work did not affect GABA levels within prisms and slices from frontoparietal cerebral cortex and hypothalamus (data not shown). GABA had no effect when it was used at low concentrations (10μM) (data not shown), but it increased significantly the GABA content of cortical (14.4 (control) vs 23.6 (GABA), nmol/mg of prot.) and hypothalamic prisms (195.6 $\pm$ 7.9 (control) vs 265.6 $\pm$ 18.6 (GABA) nmol/mg of prot.; $p < 0.01$, $n + 4$) when these preparations were incubated in the presence of 100μM GABA

Effects of $GABA_B$ receptor agonists on the synthesis of [^{3}H]GABA from [^{3}H]glutamine

The incorporation of radioactivity from [^{3}H]glutamine into [^{3}H]GABA in cortical prims was not affected by several concentration (1–100μM) of (−)baclofen (Fig. 2A). Furthermore, 3-APMPA, a more potent $GABA_B$ receptor agonist (Bon, 1996) also failed to affect the incorporation of radioactivity from [^{3}H]glutamine into [^{3}H]GABA (Fig. 2B).

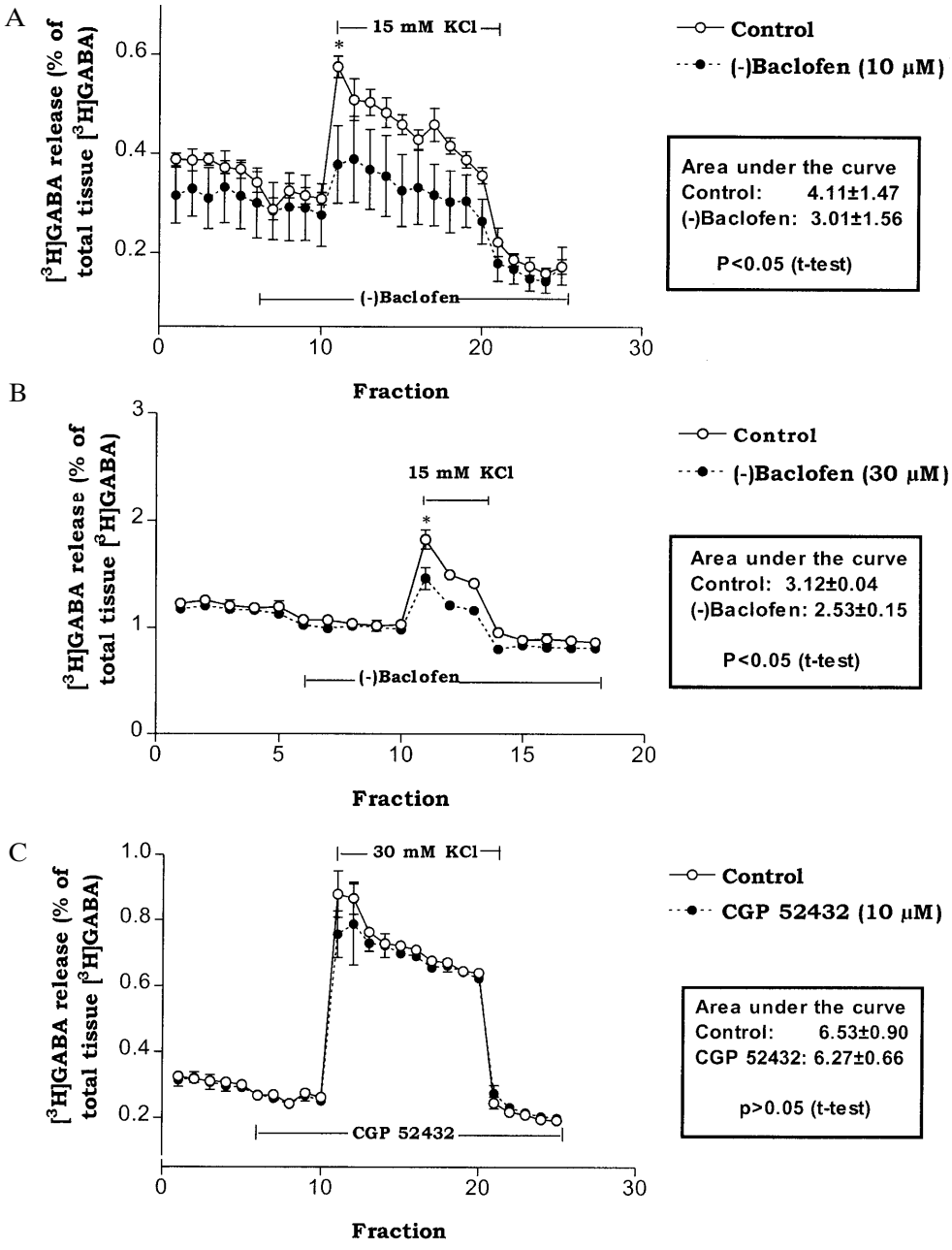

Fig. 1. Effects of (−)baclofen and CGP52432 on [^{3}H]GABA release from rat cortical prisms and synaptosomes. Prisms and synaptosomes were loaded with [^{3}H]GABA and then superfused with a Krebs-Tris medium. GABA release was evoked by exchanging the media for Krebs-Tris media containing the concentration of KCl indicated. Media containing (−)baclofen or CGP 52432 were used to superfuse the experimental chambers for the period of time indicated. [^{3}H]GABA release is expressed in percent of the radioactivity present in the preparation at the moment of its stimulation. Values are means ± SEM from 3–4 separate experiments carried out in triplicates. The area under the curve for the peak of the K^+-stimulated release of [^{3}H]GABA was obtained for each superfusion and the difference between control and experimental values was evaluated by means of the Student's "t" test. Standard error bars were omited in those points where their size was smaller than the corresponding symbols. **A** and **C**: [^{3}H]GABA release from cortical prims. **B**: [^{3}H]GABA release from cortical synaptosomes. For a complete description of the experimental procedure see Material and methods

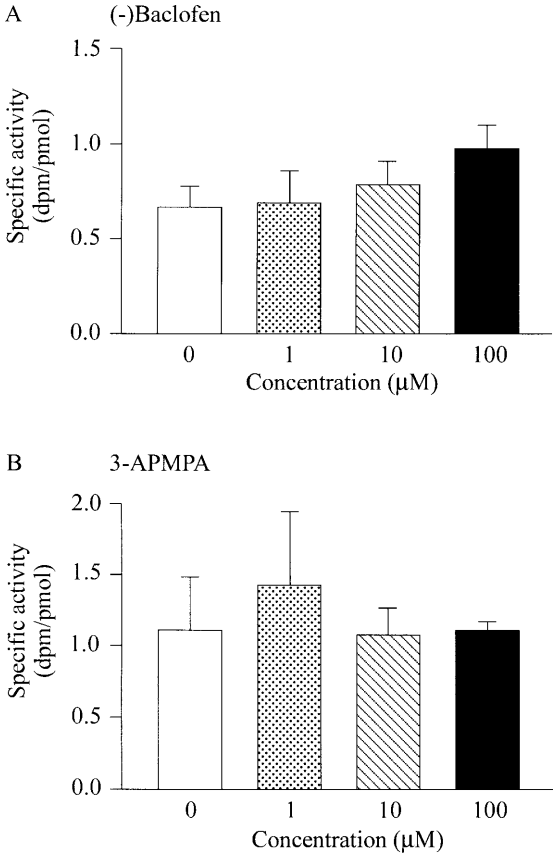

Fig. 2. Effects of (−)baclofen and 3-APMPA on the synthesis of [^{3}H]GABA from [^{3}H] glutamine in prisms from the rat frontoparietal cerebral cortex. Prisms were incubated in Krebs-Tris medium at 37° with [^{3}H]glutamine (0.33 μM; 30 Ci/mmol) for 10 min following an equilibration period of 40 min. The radioactivity incorporated into [^{3}H]GABA was determined as described under Material and methods. Values are given as means ± SEM of 3 separate experiments carried out in triplicates. For other details see text. One way ANOVA analysis showed no statistical significance

To eliminate the possibility that regional differences might be responsible for the lack of effects of $GABA_B$ receptor agonists on the synthesis of [^{3}H]GABA from [^{3}H]glutamine, the effect of (−)baclofen on the incorporation of radioactivity from [^{3}H]glutamine into [^{3}H]GABA was determined in prisms and hypothalamic slices. As shown in Fig. 3, 10 μM (−)baclofen did not modify the synthesis of [^{3}H]GABA from [^{3}H]glutamine in slices from rat hypothalamus. Similar results were obtained in hypothalamic prisms (data not shown).

As shown in Fig. 1A and B only the K^+-stimulated release of [^{3}H]GABA was modulated by (−)baclofen. Thus the effects of this $GABA_B$ receptor agonist were studied on the synthesis of [^{3}H]GABA from [^{3}H]glutamine under depolarizing conditions. The incorporation of radioactivity from [^{3}H]glutamine into [^{3}H]GABA was not modulated by (−)baclofen under any K^+ concentration (Fig. 4).

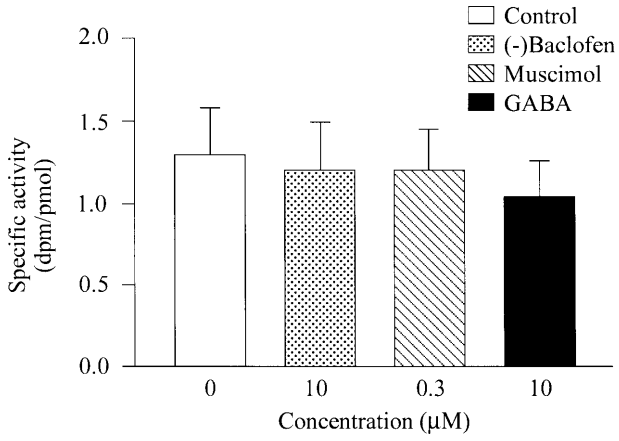

Fig. 3. Effects of (−)baclofen, muscimol and GABA on the synthesis of [^{3}H]GABA from [^{3}H]glutamine in slices from the rat hypothalamus. [^{3}H]GABA synthesis from [^{3}H]glutamine was measured in hypothalamic slices as for cortical prisms. See Fig. 2 and Material and methods for details. Values are given as means + SEM of 4 experiments carried out in triplicates. One way ANOVA analysis showed no statistical significance

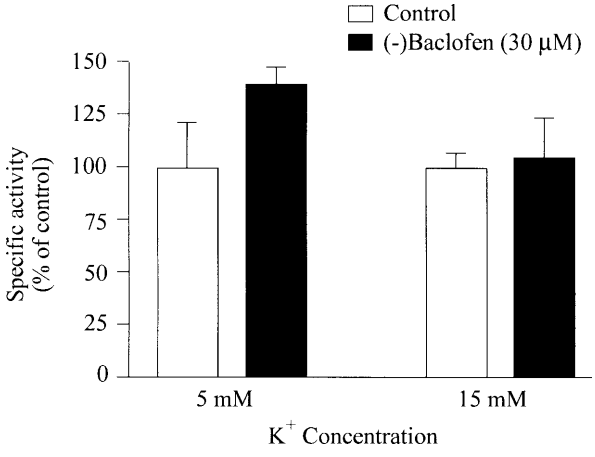

Fig. 4. Effects of (−)baclofen on the synthesis of [^{3}H]GABA from [^{3}H]glutamine in synaptosomes from the rat frontoparietal cerebral cortex. Synaptosomes were obtained by the method of Löscher et al. (1985) and resuspended in Krebs-Tris medium. Aliquots (0.3–0.7 mg protein) were equilibrated in the same medium and after 40 min the Krebs-Tris media were exchanged for fresh Krebs-Tris media containing either 5.0 mM KCl + [^{3}H]glutamine (30 Ci/mmol; 0.33 μM) or 15 mM KCl + [^{3}H]glutamine (30 Ci/mmol; 0.33 μM). The radioactivity incorporated into [^{3}H]GABA was determined as described under Material and methods. To normalize the data the results are shown as % of control values and are given as means + SEM of 3 experiments carried out in duplicates. One-way ANOVA analysis showed no statistical significance. Absolute specific activity values for the control 5 mM KCl and 15 mM KCl groups were 4.24 ± 2.03 and 2.89 ± 1.50 dpm/pmol respectively

Effects of muscimol and GABA on the synthesis of [^{3}H]GABA from [^{3}H]glutamine

In order to explore a possible modulation of GABA synthesis by presynaptic $GABA_A$ autoreceptors (Mitchell and Martin, 1978; Floran et al., 1988; Hashimoto and Kuriyama, 1997), the effects of the $GABA_A$ agonist muscimol (Simmonds, 1983) on the incorporation of radioactivity from [^{3}H]glutamine into [^{3}H]GABA were also studied. In addition, the effects of GABA on its own synthesis from [^{3}H]glutamine were evaluated. Fig. 3 shows that neither muscimol (10μM) nor GABA (10μM) affected the synthesis of [^{3}H]GABA from [^{3}H]glutamine in slices of rat hypothalamus. However, there was a trend for a decrease in [^{3}H]GABA synthesis both in hypothalamic and in cortical prisms when higher (100μM) GABA concentrations were used. In the hypothalamus the control and GABA group values were 0.5 ± 0.17 and 0.35 ± 0.08 dpm/pmol respectively; n = 4. In the cerebral cortex the respective values were 1.43 and 0.92 dpm/pmol (one experiment in quintuplets).

Effects of $GABA_B$ and $GABA_A$ receptor agonists on GAD activity

To further study a possible modulation of GABA synthesis by presynaptic GABA autoreceptors GAD activity was measured within cortical and hypothalamic prisms in the presence of $GABA_B$ and $GABA_A$ receptor agonists. Neither, (−)baclofen, muscimol, or GABA showed any modulatory effect on cortical GAD activity (Fig. 5). Identical results were also obtained in prisms from hypothalamus (data not shown).

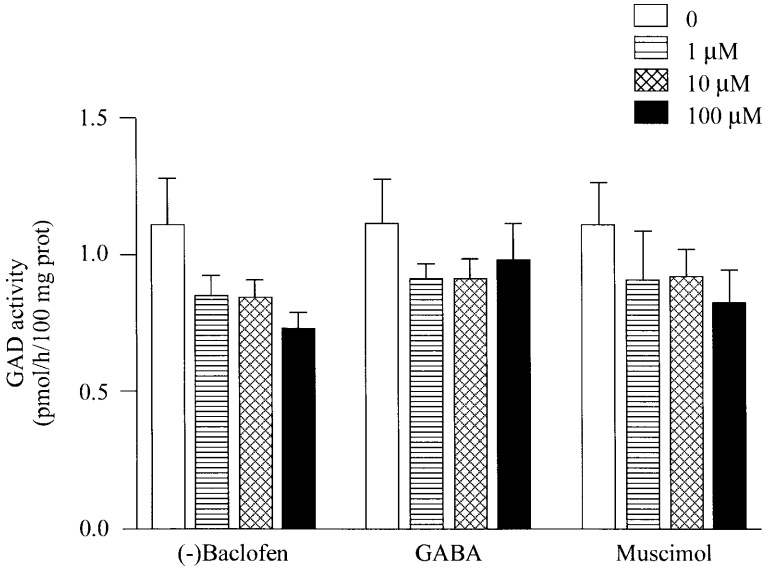

Fig. 5. Effects of (−)baclofen, GABA and muscimol on GAD activity in prisms from the rat frontoparietal cerebral cortex. GAD activity was measured radioisotopically by measuring the evolution of $^{14}C0_2$ from [1^{14}C]glutamic acid in the presence of different concentrations of either (−)baclofen, GABA or muscimol. Data are the mean ± SEM of 5 different experiments carried out in triplicates. See Material and methods for methodological details. One-way ANOVA analysis showed no statistical significance

Effects of CGP 52432 on [^{3}H]GABA release, GAD activity and [^{3}H]GABA synthesis from [^{3}H]glutamine

The rule out the possibility that the lack of effect of (−)baclofen on GABA synthesis resulted from a saturation of $GABA_B$ autoreceptors by GABA release under basal conditions (Waldmeier et al., 1993) which may have prevented any further autoreceptor activation by (−)baclofen, we studied the effects of CGP 52432, a selective $GABA_B$ receptor antagonist (Lanza et al., 1993), on [^{3}H]GABA release, [^{3}H]GABA synthesis from [^{3}H]glutamine and GAD activity in cortical prisms. Furthermore, in all the experiments described in this section a 30 mM K^+ concentration was used in an attempt to induce a compensatory increase in GABA synthesis as a consequence of a deeper depletion in the releasable pool of GABA. As indicated in Fig. 1C, CGP 52432 did not affect [^{3}H]GABA release. Likewise, [^{3}H]GABA synthesis from [^{3}H]glutamine (Fig. 6A) and GAD activity (Fig. 6B) were not modified.

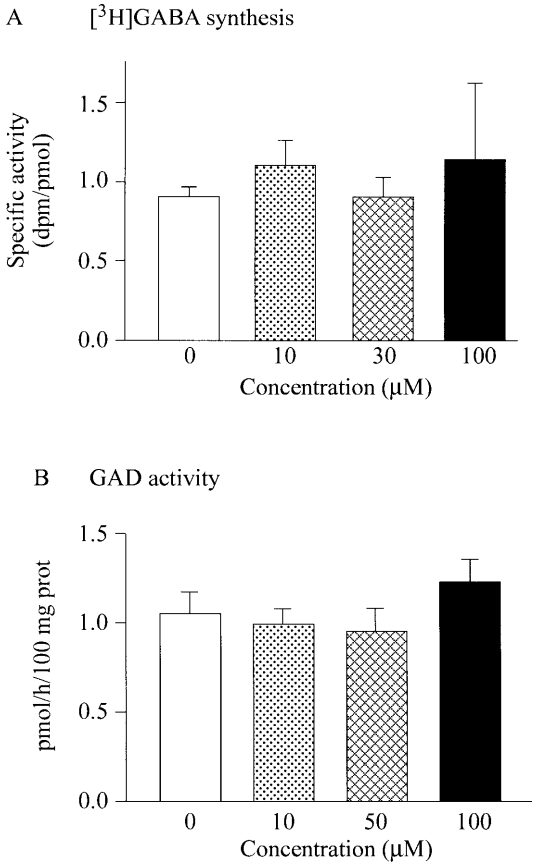

Fig. 6. Effects of CGP 52432 on the synthesis of [^{3}H]GABA from [^{3}H]glutamine and on GAD activity in prisms from the rat frontoparietal cerebral cortex. [^{3}H]GABA synthesis and GAD activity were measured as described in Material and methods. Values are given as means ± SEM of 5 different experiments carried out in triplicates. No statistical significant differences (ANOVA analysis) were found on the effects of CGP 52432 on both [^{3}H]GABA synthesis from [^{3}H]glutamine and GAD activity

Discussion

As documented above a tight coupling between neurotransmitter release and synthesis seems to occur within the aminergic neuronal systems (Carlsson, 1975; Meller et al., 1990; Westernik et al., 1990; Goldstein, 1995; Elsworth and Roth, 1996; Gainetdinov et al., 1996). In these systems, and particularly within the dopaminergic one, the activation of a single or a set of different presynaptic metabotropic autoreceptor subtypes leads to a decrease in both neurotransmitter release and synthesis (Goldstein, 1995; Elsworth and Roth, 1996; Gainetdinov et al., 1996; Mercuri et al., 1997; Whetzel et al., 1997; Schaffer and Levant, 1998). In this paper, we studied in prisms, slices and synaptosomes from two brains regions the effects of several concentrations of two $GABA_B$ receptor agonists and one antagonist on both [^{3}H]GABA release and GABA synthesis. The purpose was to ascertain if the same type of coupling exists within the gabaergic system. Since GABA synthesis is a highly compartmentalized process (Van den Berg et al., 1977; Schousboe et al., 1997) and glutamine seems to be a major GABA precursor (Paulsen et al., 1988) we measured, as indexes of GABA synthesis, the incorporation of radioactivity from [^{3}H]glutamine into [^{3}H]GABA as well as the GAD activity within brain preparations which still retain a great deal of compartmentation (Balázs et al., 1970).

In agreement with work published by many laboratories (i.e. Floran et al., 1988; Waldmeier et al., 1992; Lanza et al., 1993) (−)baclofen decreased the K^+ stimulated [^{3}H]GABA release from cortical prisms (Fig. 1A) and synaptosomes (Fig. 1B). However it failed to influence [^{3}H]GABA synthesis from [^{3}H]glutamine in cortical (Fig. 2A) and hypothalamic prisms (data not shown) as well as in hypothalamic slices (Fig. 3). Furthermore 3-APMPA (1–100μM), a more potent $GABA_B$ receptor agonist (Bon et al., 1996) also failed to modulate [^{3}H]GABA synthesis from [^{3}H]glutamine in cortical prisms. In line with these last results (−)baclofen (1–100μM) did not affect GAD activity in cortical (Fig. 5) and hypothalamic prisms (data not shown). The possibility that either regional differences might be involved in the lack of effects of (−)baclofen on GABA synthesis, or that $GABA_B$ agonists only modulate GABA synthesis under conditions of stimulated GABA release seems unlikely, since similar negative results were observed in cortex and hypothalamus and at low (5 mM) or high (15 mM) K^+ concentrations (Fig. 4). An alternative explanation for the lack of effects of (−)baclofen on GABA synthesis in slices and prisms might be that in our conditions $GABA_B$ autoreceptors are already fully activated by basal GABA release (Waldmeier et al., 1993). However, the fact that GABA synthesis is not affected by (−)baclofen in synaptosomes (Fig. 4) in which the GABA released under basal conditions is highly diluted and washed by the superfusion and that CGP 52432, a selective $GABA_B$ receptor antagonist (Lanza et al., 1993; see however Waldmeier et al., 1994), did not affect neither [^{3}H]GABA release (Fig. 1C), nor GABA synthesis (Fig. 6) renders this possibility also unlikely.

An apparent lack of coupling between $GABA_B$ receptor activation and the rate of GABA synthesis has been already reported by Potashner (1997), who found that 4μM baclofen did not modify the concentration and the labeling of [^{14}C]GABA from [^{14}C]glucose. However, the issue of the possible coupling between GABA release and synthesis is far from settled, since in those experiments only one baclofen concentration was tested; moreover baclofen failed to modify significantly the electrically stimulated GABA release, as it usually does (this paper and i.e. Floran et al., 1988; Raiteri et al., 1989; Waldmeier and Baumman, 1990). The results of the systematic study reported in this paper thus suggest the lack of a coupling between the GABA release and its synthesis.

Since it is has been reported that $GABA_A$ autoreceptors modulate GABA release (Mitchell et al., 1978; Floran et al., 1988; Hashimoto and Kuriyama, 1997), we explored the possibility that GABA synthesis may be modulated by the $GABA_A$ receptor agonist muscimol. Our results show that $GABA_A$ autoreceptor mechanisms are not involved in the regulation of GABA synthesis, since muscimol failed to affect both [^{3}H]GABA synthesis from [^{3}H]glutamine in slices of hypothalamus (Fig. 3), and GAD activity within cortical (Fig. 5) and hypothalamic (data not shown) prisms. It is clear however that more studies are needed before a modulatory effect of $GABA_A$ autoreceptors on GABA synthesis can be discarded. GABA seems to be also unable to modify its own synthesis, since only a trend for a decrease in the labeling of [^{3}H]GABA from [^{3}H]glutamine was found at the highest (100μM) GABA concentration used.

In conclusion, the results of these paper, based on the pharmacological actions of (−)baclofen, 3-APMPA and CGP 52432, support a lack of coupling between GABA release and synthesis mediated through a pharmacologically identical $GABA_B$ autoreceptor. However, considerable evidence suggests that different subtypes of DA D_2 receptors couple the activation of DA D_2 autoreceptors to modifications in either their release or synthesis (Goldstein, 1995; Elsworth and Roth, 1996; Gainetdinov et al., 1996; O'Hara et al., 1996; Mercuri et al., 1997; Whetzel et al., 1997; Schaffer and Levant, 1998). Likewise, recent expression cloning experiments have shown the existence of at least two metabotropic $GABA_B$ receptors (Kaupmann et al., 1997) which may have several splice variants. Therefore, it is conceivable that GABA release and synthesis may be coupled to different subtype of metabotropic $GABA_B$ autoreceptor.

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