ELSEVIER

Carbamazepine-Induced Upregulation of Adenosine A_1-Receptors in Astrocyte Cultures Affects Coupling to the Phosphoinositol Signaling Pathway

Knut Biber, Ph.D., Bernd L. Fiebich, Ph.D., Peter Gebicke-Härter, Ph.D., and Dietrich van Calker, Ph.D., M.D.

The anticonvulsant and antibipolar drug carbamazepine (CBZ) is known to act as a specific antagonist at adenosine A_1-receptors. After a 3-week application of CBZ, A_1-receptors are upregulated in the rat brain. We have investigated the consequences of this upregulation for the A_1-receptor-mediated signal transduction in primary astrocyte cultures from different regions of the rat brain. CBZ treatment for 10 days had no effect on adenosine A_1-receptor mRNA expression in cultures with high basal A_1-receptor mRNA levels, but increased A_1-receptor mRNA in cultures exhibiting low basal A_1-receptor mRNA levels. This upregulation of A_1-receptor mRNA was accompanied by an upregulation or induction of A_1-receptor-mediated potentiation of PLC activity, a property that was not found in these cultures before CBZ treatment. Thus, CBZ treatment for 10 days induces a new quality of adenosine A_1-receptor-mediated signal transduction in cells that express low basal A_1-receptor numbers.

[Neuropsychopharmacology 20:271–278, 1999]

KEY WORDS: *Affective disorders; Carbamazepine; Adenosine A_1 receptors; Signal transduction; mRNA expression; Chronic treatment*

The anticonvulsant drug carbamazepine (CBZ) is known to exert antimanic and prophylactic effects in the treatment of manic-depressive disorders that are clinically equivalent to the well-known properties of lithium salts. CBZ may even have advantages as compared to lithium in the treatment and prophylaxis of schizoaffective and rapid cycling bipolar disorders (for review see Solomon et al. 1995). Among the various pharmacological effects of CBZ, its activity as a specific antagonist at adenosine A_1 receptors (van Calker et al. 1991; Biber et al., 1996) may be particularly important, because this property could lead to alterations in the responsiveness of signal transduction systems in the brain (van Calker et al. 1998), a mechanism believed to be instrumental also to lithium's beneficial effects in affective disorders (for review see Manji et al. 1995).

Adenosine A_1- and A_2-receptor subtypes were originally distinguished by their differential effects on adenylyl cyclase (van Calker et al. 1978, 1979), but more recent findings have revealed coupling of adenosine receptors to other signal transduction systems including phospholipase C (PLC) (Miller and Hoffman 1994; Fredholm et al. 1994). Very often, the coupling of adenosine A_1-receptors to PLCβ is synergistic with the stim-

From the Department of Psychiatry (BLF, PG-H, DvC), University of Freiburg, Freiburg, Germany; and Department of Medical Physiology (KB), University of Groningen, Groningen, The Netherlands.

Address correspondence to: Dietrich van Calker, Ph.D., M.D., Department of Psychiatry, University of Freiburg, Hauptstr. 5, D-79104 Freiburg, Germany.

Received 11 January 1998; revised 25 May 1998; accepted 10 June 1998.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/99/$–see front matter
PII S0893-133X(98)00059-1

ulation evoked by other receptors, such as α_1-adrenergic receptors (El-Etr et al. 1989; Biber et al. 1996, 1997), histamine H_1-receptors (Dickenson and Hill 1994), muscarinic receptors (Biden and Browne 1993), or thyrotropin receptors (Okajima et al. 1995). There are, however, also reports suggesting direct activation of PLC by adenosine A_1-receptors (Freund et al. 1994; Murthy and Makhlouf 1995).

We recently reported that the stimulatory effects of adenosine A_1-receptors on PLC signaling in primary astrocytes from different brain regions depend upon the expression level of adenosine A_1-receptor mRNA and protein. Adenosine A_1-receptor mediated PLC activation was only evident in cultures expressing high receptor numbers but was absent in cultures expressing low numbers of adenosine A_1-receptors (Biber et al. 1997). We, therefore, hypothesized that upregulation of A_1-receptors should alter their coupling to second messenger systems. Little is known about the physiological regulation of adenosine A_1-receptor expression, but available evidence indicates an upregulation of receptor number after chronic treatment with receptor antagonists (Shi et al. 1993; Von Lubitz et al. 1994b). Thus, chronic treatment (3 weeks) with the antagonist CBZ, also results in upregulation of A_1-receptors in the brain (Daval et al. 1989). We report here that in cultured astrocytes with low basal expression of A_1-receptors mRNA, upregulation of receptor mRNA by CBZ treatment for 10 days induces their coupling to PLC; whereas, mRNA expression and signal transduction of adenosine A_1-receptors in cultures with high basal mRNA levels remains unaltered.

MATERIALS AND METHODS

Materials

Reagents were purchased from the following sources: cyclopentyladenosine (CPA), phenylephrine (PE), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and isoproterenol from Research Biochemical Inc. (RBI) and cyclic AMP-RIA Kit from Immuno Biological Laboratories (IBL), all distributed by Biotrend (Köln, Germany); Dulbecco's modified Eagle's medium (DMEM) and carbamazepine (CBZ) from Sigma (Deisenhofen, Germany); fetal calf serum from Boehringer (Mannheim, Germany); scintillation fluid (rotiszint ecoplus) from Roth (Karlsruhe, Germany); dowex anion exchanger (formiate form AG 1 × 8) from Bio Rad (München, Germany); [^{3}H]-*myo*-inositol and [^{32}P]dCTP from Amersham & Buchler (Braunschweig, Germany); Bst E II from Pharmacia (Freiburg, Germany); Dynabeads from Dynal AG (Hamburg, Germany); TA cloning kit from Invitrogen (Leek, The Netherlands); random priming kit Prime IT II from Stratagen (Heidelberg, Germany); cDNA probe encoding β-actin was a generous gift from Dr. G. Finkenzeller (Institute for Tumor Biology, Freiburg, Germany).

Cell Cultures

Astrocyte cultures were established as described previously (Gebicke-Härter et al. 1989). In brief, rat brains were dissected out of newborn Wistar rat pups (< 1d) under sterile conditions, and various brain regions (cortex, hippocampus, striatum, tegmentum, thalamus, and cerebellum) were isolated. Brain tissues were gently dissociated by trituration in Dulbecco's phosphate buffered saline and filtered through a cell strainer (70 μm Ø, Falcon) into Dulbecco's modified Eagle's medium (DMEM). After two washing steps (200 × *g* for 10 min), cells were seeded into 24-well-dishes (Falcon) (5×10^5 cells/well). For total RNA and mRNA preparations, cells were grown in 6-well dishes (Falcon) (2×10^6 cells/dish) and standard dishes (Falcon, 10 cm Ø) (8×10^8 cells/dish), respectively. Cultures were maintained for 4 weeks in DMEM containing 10% fetal calf serum with 0.01% penicillin and 0.01% streptomycin in a humidified atmosphere (5% CO_2) at 37°C. Culture medium was changed on the 2nd day after preparation and every 6 days thereafter.

Cyclic AMP-Determination

Four-week-old cultures were washed three times with 500 μl incubation buffer (118 mm NaCl, 4.7 mm KCl, 3 mm CaCl, 1.2 mm $MgSO_4$, 1.2 mm KH_2PO_4, 0.5 mm EDTA, 10 mm glucose, and 20 mm HEPES; pH 7.4). Cells were then stimulated at 37°C in the same buffer with the β-adrenergic receptor agonist isoproterenol (1 μm) in the presence or absence of CPA. After 10 min, the buffer was removed, and the reaction was stopped by addition of 400 μl ice-cold ethanol (70%). Dishes were incubated on ice for 30 min, and the supernatants were harvested. 10 μl of each sample was dried in a speed vac centrifuge (Bachhofer, Reutlingen, Germany) and redissolved in 500 μl sample buffer (cAMP-RIA Kit IBL). Cyclic AMP levels were determined according to the manufacturer's protocol.

Inositolphosphate Determination

Four-week-old cultures were labeled for 24 h with 1 μCi [^{3}H]-*myo*-inositol in 250 μl of culture medium. After three washings with 500 μl incubation buffer, cells were incubated for 15 min at 37°C in the same buffer supplemented with 10 mm LiCl and stimulated for 20 min with the α_1-adrenergic receptor agonist phenylephrine (100 μm) in the presence or absence of CPA. The reaction was stopped by addition of ice-cold TCA (100% w/v) up to a final concentration of 10% TCA. Dishes were incubated on ice for 30 min. Separation of inositolphos-

phates was performed on Dowex anion exchange columns (Formiate Form AG 1 × 8) as described previously (van Calker et al. 1987). In brief, TCA was extracted with diethylether (three times), and samples were neutralized to pH 7 with 5 mm disodium tetraborate. Samples were loaded on Dowex columns, and after two washing steps (10 ml water, 10 ml 50 mm disodium tetraborate/60 mm ammonium formate), inositolphosphates were eluted with 2 ml of 1 m ammonium formate and 0.1 m formic acid. Samples were mixed with 6 ml of liquid scintillation fluid and counted. Determinations were performed in triplicate.

Total RNA Extraction

Cells were lysed in guanidinium isothiocyanate/mercaptoethanol (GTC) solution (250 μl/well). Two samples were pooled to a final volume of 500 μl lysate and total RNA was extracted according to Chomczynski and Sacchi (1987).

Cloning of the cDNA Probe for Adenosine A_1 Receptors

Total RNA was reverse transcribed and PCR experiments were carried out as described previously (Biber et al. 1997). Sequences of oligonucleotide primer pairs and PCR conditions: Adenosine A_1-receptor, # 55 5<<-ATTGCCTTGGTCTCTGTGC and #690 5<<-CAGCTCCTTCCCGTAGTAC, annealing temperature 59°C, 35 cycles. The PCR product was cloned using the TA cloning kit (Invitrogen) and sequenced by ALF (Pharmacia Biotech, Germany) revealing 100% homology to the known cDNA sequence for rat A_1-receptor (Mahan et al. 1991). 50 μg of the vector containing the A_1 receptor cDNA was digested by Bst E II, and the fragments were separated on a preparative agarose gel. The 300 bp fragment of the A_1-receptor cDNA was used for Northern analysis.

mRNA Extraction and Northern Analysis

Cells were lysed in 500 μl lysis/binding buffer (Dynal), and mRNA was extracted with 150 μl Dynabeads according to the manufacturers protocol. mRNA (2–3 μg) was eluted in 15 μl water, separated by agarose-formaldehyde gel electrophoresis, blotted onto positively charged nylon membranes (Pharmacia, Freiburg), and cross-linked by exposure to 120°C for 30 min. As a marker 15 μg of total RNA was run on the same gel and stained with ethidium bromide to determine the bands for 18 s and 28 s ribosomal RNA. The filters were prehybridized in 50% formamide, 0.25 m Na-phosphate buffer, pH 7.2, 0.25 m NaCl, 10 mm EDTA, 200 μg/ml salmon sperm DNA and 7% SDS at 43°C for 2 h. cDNA probes were labeled with 50 μCi [^{32}P]dCTP (Amersham & Buchler, Braunschweig, Germany) using a random priming kit from Stratagene (Heidelberg, Germany). Unincorporated nucleotides were removed using a nucleotide removal kit from Qiagen. Overnight hybridization was performed at 43°C, adding the radiolabeled probe to the prehybridization buffer. Membranes were washed in 2 × SSC/0.1% SDS (3 × 20 min) at 60°C and exposed to Kodak XAR film at −80°C for adequate periods of time. For rehybridization, probes were removed by boiling the filter at 95°C in distilled water. Experiments were carried out in triplicate.

RESULTS

Primary astrocyte cultures from different regions of the rat brain were treated for 10 days with CBZ (50 μm). Then cultures were analyzed for (1) expression of adenosine A_1-receptor mRNA (2) adenosine A_1-receptor mediated inhibition of cyclic AMP synthesis; (3) adenosine A_1-receptor-mediated potentiation of PLC activation.

Expression of Adenosine A_1-Receptor mRNA

Adenosine A_1-receptor mRNA expression was investigated by Northern blot analysis. The results obtained for untreated cultures were similar to those previously obtained by semiquantitative RT–PCR experiments (Biber et al. 1997). Signals for adenosine A_1-receptor mRNA were found in mRNA preparations from astrocytes cultured from all brain regions investigated. Higher levels were observed in hippocampal, cortical, tegmental, and thalamic cultures (as shown in Figure 1A for hippocampal cultures); whereas, lower levels were found in cultures from whole brain, striatum, and cerebellum (as shown for cultures from cerebellum in Figure 1B). Remarkably, treatment with CBZ did not substantially alter the adenosine A_1-receptor mRNA level in cultures with high expression levels (hippocampus, cortex, thalamus, and tegmentum) (Figure 1A). However, a pronounced upregulation of adenosine A_1-receptor mRNA was observed after CBZ treatment in cultures from whole brain, striatum, and cerebellum (Figure 1B).

Inhibition of Cyclic AMP

As already reported, stimulation of adenosine A_1-receptors leads to inhibition of the isoproterenol-induced increase of cyclic AMP (van Calker et al. 1978). The maximal inhibitory effect was approximately 50% and independent of the brain region from which the astrocytes had been cultured (Biber et al. 1997). Ten-day treatment with the A_1-receptor antagonist CBZ did not alter these inhibitory effects. No significant changes of the concentration response curves after carbamazepine treatment were observed in cultures with high or with

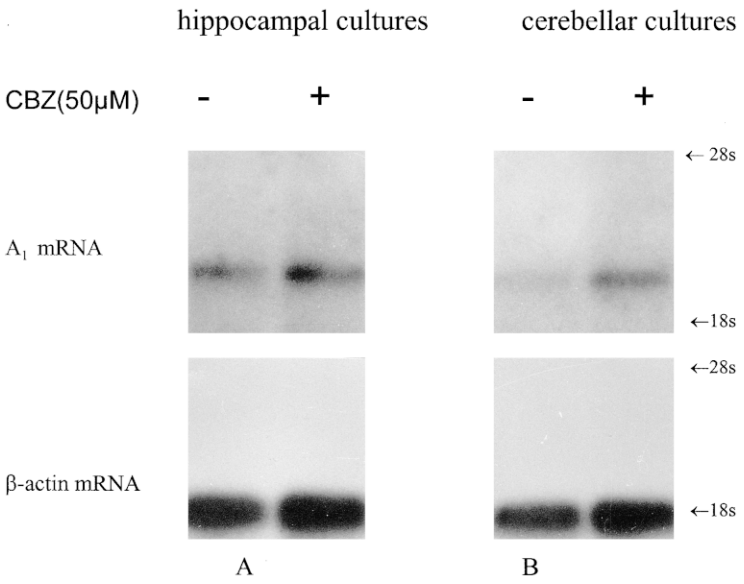

Figure 1. Northern blot analysis of adenosine A_1-receptor mRNA expression from primary astrocyte cultures with (+) or without (−) treatment (10 days) with CBZ (50 μm). **(A)** Results obtained from hippocampal cultures (similar results were obtained in cultures from cortex, thalamus and tegmentum). **(B)** Results obtained from cerebellar cultures (similar results were obtained in cultures from whole brain and striatum). Control hybridizations were performed with a probe for β-actin. Results shown here are parts taken from the same northern blot. Arrows indicate 18 s and 28 s ribosomal RNA, which had been determined by running of 15 μg total RNA in the same Northern. Three independent repetitions of the experiments gave similar results.

low A_1 mRNA expression (as shown in Figure 2 for cultures from hippocampus and cerebellum, respectively).

Potentiation of PLC-Activation

Astrocyte cultures established from thalamus, tegmentum, cortex, and hippocampus, expressing high levels of adenosine A_1-receptors, exhibit adenosine A_1-receptor-mediated potentiation of agonist-activation of PLC (Biber et al. 1997). Ten-day treatment of these cultures with CBZ did not significantly influence the signaling of adenosine A_1-receptors to PLC (Figure 3). As shown previously, astrocyte cultures established from whole brain, cerebellum, and striatum show only moderate (whole brain) or little, if any, effect of adenosine A_1-receptor stimulation on PLC activity (Biber et al. 1997). Ten-day treatment with CBZ, however, significantly increased the adenosine A_1-receptor-mediated potentiation of PLC activity in cultures from whole brain (Figure 4) and induced an adenosine A_1-receptor-mediated potentiation in cultures from striatum and cerebellum (Figure 4).

DISCUSSION

The results of the present study provide evidence that upregulation of adenosine A_1-receptors can alter their coupling to signal transduction systems. This is concluded from the following findings.

Primary astrocyte cultures from different brain regions can be subdivided into two groups according to the expression levels of adenosine A_1-receptor mRNA: those with high expression (cultures from hippocampus, cortex, thalamus, and tegmentum) and those with low (cerebellum, striatum) expression of A_1-receptor mRNA. Both determination of mRNA by Northern blot analysis (this paper) and with RT–PCR (Biber et al. 1997) revealed similar results.

Ten-day treatment with 50 μm CBZ (which displays the same A_1 antagonistic activity as 100 nm DPCPX [Biber et al. 1996]) induced a pronounced upregulation of A_1-receptor mRNA only in cultures with low expression (cerebellum, striatum, and whole brain). This upregulation of A_1-receptor mRNA was accompanied by an induction (cerebellum and striatum) or upregulation (whole brain) of adenosine A_1-receptor-mediated potentiation of PLC activation. On the other hand, CBZ treatment had no effect on mRNA expression and A_1-receptor signaling in cultures, which already at basal conditions exhibited a high A_1-receptor mRNA content and an A_1-receptor-dependent PLC potentiation.

We have shown previously that in these cultures, A_1-receptor mRNA concentration strictly correlates with receptor-binding properties. Thus, we detected in high expression cultures (hippocampus, cortex, thalamus, and tegmentum) B_{max} values of 24 ± 7 fmol/mg and in low expression cultures (striatum and cerebellum) B_{max} values of ⩽ 5 fmol/mg (which was near the detection limit) (Biber et al. 1997). We verified in one experiment that, after CBZ treatment, not only adenosine A_1-receptor mRNA is upregulated but also the receptor protein in striatal and cerebellar cultures as determined by radioligand-binding studies. Thus, CBZ treatment induced an increase of B_{max} values up to those observed in the high-affinity group. Therefore, potentiation of PLC activation by A_1-receptors relies on the number of A_1-receptors occupied by agonists. This is most likely explained by the bifurcating nature of G-protein signaling; that is, that the activation of *one* Gi/o protein by *one* adenosine A_1-receptor gives rise to *two* different signal

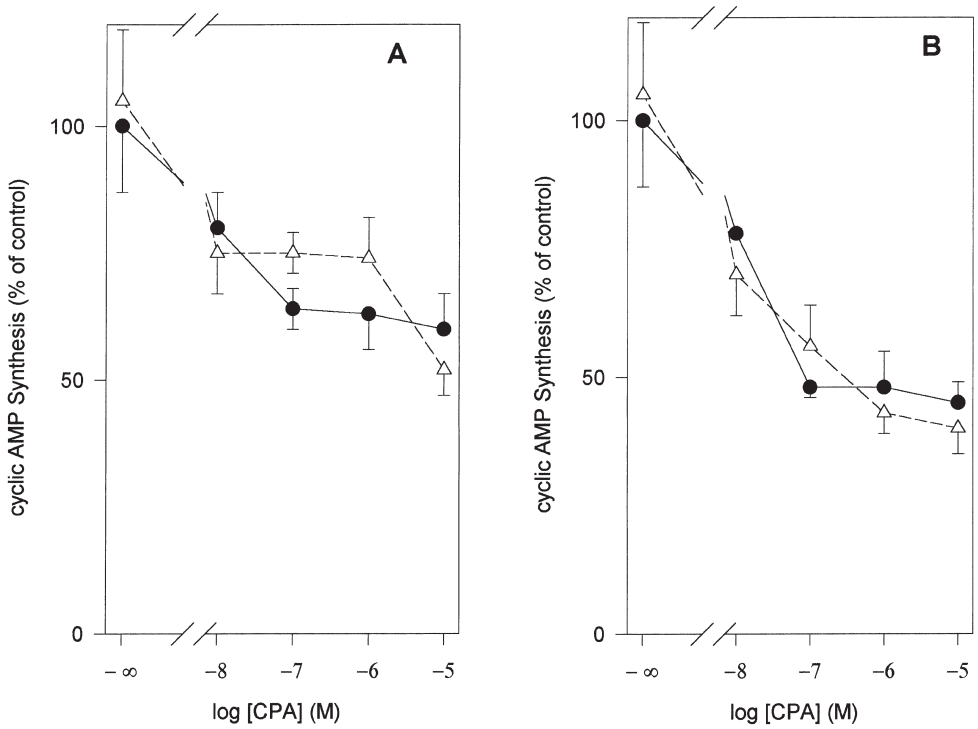

Figure 2. Lack of effect of CBZ treatment on the inhibitory effect of CPA on the isoproterenol (1 μm)-induced accumulation of cAMP in primary astrocyte cultures from cerebellum **(A)** and hippocampus **(B)**. Cultures with (△) or without (●) treatment (10 days) with CBZ (50 μm). Control level of cyclic AMP in unstimulated cells: 0.5 ± 0.4 pmol/well; cyclic AMP levels in isoproterenol (1 μm) stimulated cells were between 48 ± 13 pmol/well and 65 ± 14 pmol/well depending on the brain region (values are given as means of three independent experiments ± SD). Similar results were obtained from experiments performed with cultures from cortex, tegmentum, thalamus striatum, and whole brain.

transducers (α and βγ subunits) with different affinities for their target enzyme. It is well known that α subunits have 10 to 100-fold higher affinities to their target enzymes than βγ subunits (for review see Müller and Lohse 1995). The high affinity of α subunits for adenylyl cyclase allows an inhibition already at comparably low numbers of α subunits being activated in cultures with low A_1-receptor mRNA and protein. In contrast, because of their low affinity to PLC, more free βγ subunits are required to activate PLC, which are released in cultures with high A_1-receptor expression (Biber et al. 1997). The results presented here support this notion, because upregulation of A_1-receptor mRNA upregulates or even induces an A_1-receptor mediated PLC activation but leaves unaltered the inhibition of adenylyl cyclase by A_1-receptors.

Several other mechanisms could be envisaged to explain the CBZ-induced increase in the extent of PLC-activation; for example, changes in G-protein content, induction of specific G-protein subunits, changes in levels of PLC, or changes in inositol phospholipid availability. However, we have shown previously that G-protein and PLC contents of all cultures are comparable (measured by a direct activation of Gi/o-proteins by mastoparan) (Biber et al. 1997). Moreover, there are no data available at the moment indicating that CBZ might have any effect on G-protein and/or PLC activity or expression or inositol phospholipid metabolism.

Adenosine A_1-receptor up and down regulation by chronic treatment with adenosine A_1-receptor antagonists and agonists, respectively, has been reported to modulate cerebral ischemia, seizures, spatial learning, and memory (Von Lubitz et al. 1993a,b, 1994a,b), but little is known about the physiological consequences of adenosine A_1-receptor upregulation for their coupling to signal transduction systems. In general, there are only few reports in the literature that examine the consequences of changes in receptor expression for their signal transduction. Upregulation of receptor number may result in an increased signal transduction, as shown for the adenosine A_1-receptor-mediated inhibition of adenylyl cyclase in chick atrial myocytes (Liang and Hirsch 1993) or the stimulation of adenylyl cyclase by vasoactive intestinal peptide receptors in leukocytes (Wiik 1991). Other reports, however, indicate that receptor upregulation did not enhance signal transduction events, as shown for bradykinin receptor 2 in human glomerular podocytes (Costenbader et al. 1997) or

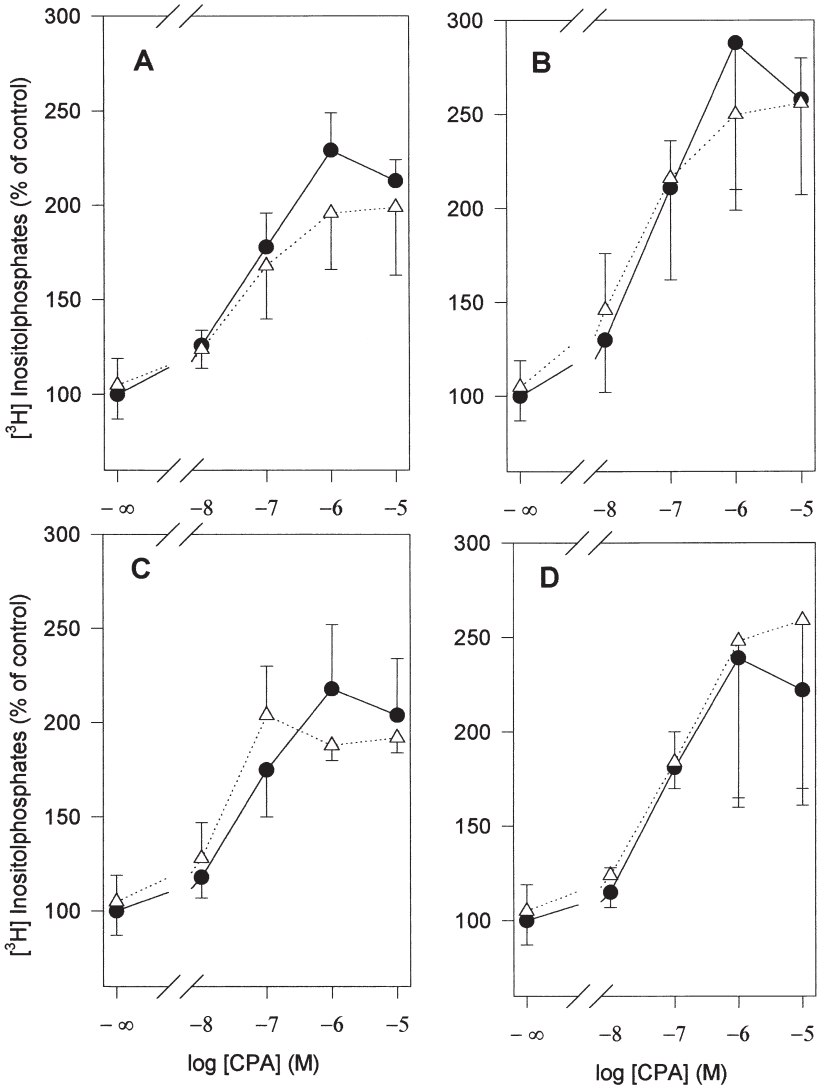

Figure 3. Lack of effect of CBZ treatment on the potentiation by CPA of inositolphosphate accumulation in astrocyte cultures evoked by phenylephrine (100 μm) (=100%) from cortex **(A)**, hippocampus **(B)**, tegmentum **(C)**, and thalamus **(D)**. Cultures with (△) or without (●) treatment (10 days) with carbamazepine (50 μm). Determinations were performed in triplicate and values given are means of three independent experiments ± SD.

muscarinic acetylcholin receptors in esophageal sphincter muscle (Keshavarzian et al. 1992). To our knowledge, this is the first report indicating that upregulation of a receptor induces a new quality of signal transduction.

Several treatments of affective disorders (CBZ treatment, sleep deprivation, and electroconvulsive therapy [ECT]) lead to an upregulation of adenosine A_1-receptors in brain (for review see Durcan and Morgan 1990; Williams 1995). Our results indicate that this upregulation may lead to an increase or induction of Gi/o protein βγ subunit-mediated actions. G protein βγ subunits are known to have several intracellular effects (for review see Müller and Lohse 1995). Particularly, it is becoming obvious that βγ subunits modify currents through several ion channels in the brain (Ma et al. 1997; Qin et al. 1997; Herlitze et al. 1996; Ikeda 1996; Wickman et al. 1994). It is, therefore, tempting to speculate that upregulation of adenosine A_1-receptors in brain during the treatment of affective disorders may influence neuronal excitability by means of an increased or induced βγ subunit-mediated influence on neuronal ion fluxes and that this effect on neuronal activity may participate in the therapeutic effects of CBZ, sleep deprivation, or ECT.

In conclusion, we have provided evidence that a 10-day treatment with CBZ upregulates A_1-receptor mRNA expression in primary astrocyte cultures from brain regions where receptor expression is low; whereas, receptor mRNA expression in cultures from brain regions where receptor expression is high were unaffected. Upregulation of adenosine A_1-receptors had no influence on their inhibitory effect on cyclic AMP synthesis but increased or even induced adenosine A_1-receptor-mediated potentiation of PLC activity. These results support the hypothesis that the coupling of adenosine A_1-receptors to PLC is determined by their expression level in the cell. They provoke the question as to whether the well-known alterations in the sensivity of receptors for neurotransmitters that are induced by various pharmacological and nonpharmacological ther-

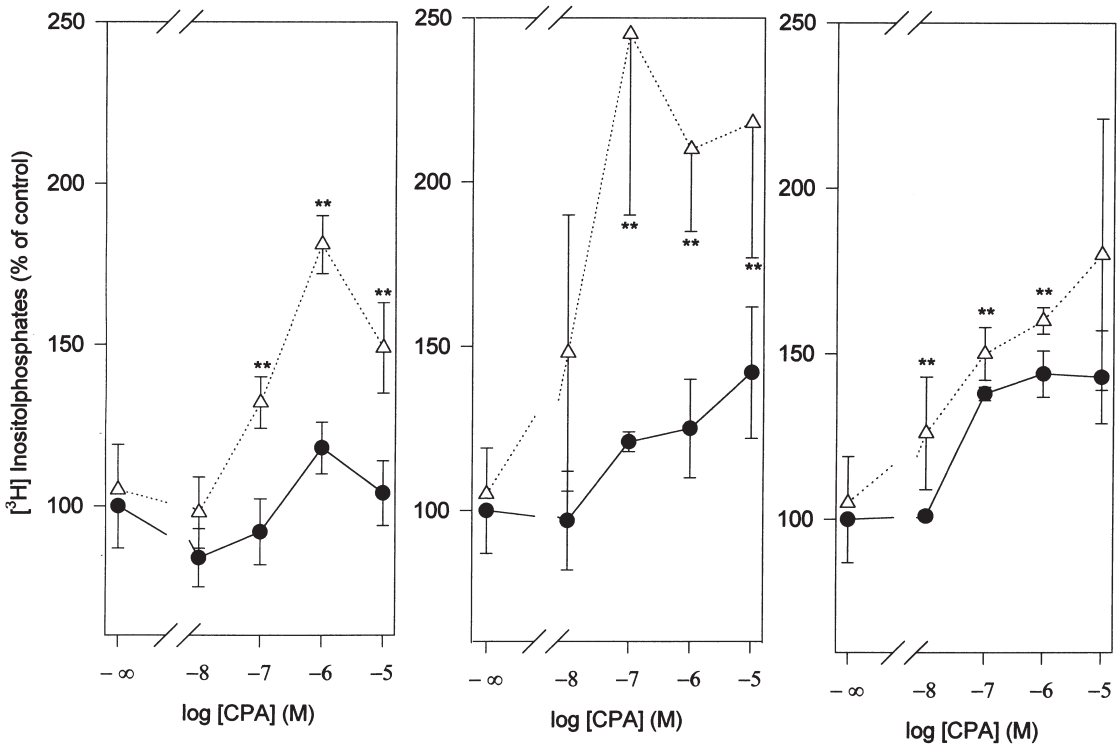

Figure 4. Effects of CBZ treatment on the potentiation by CPA of inositolphosphate accumulation evoked by phenylephrine (100 μm) (=100%) in astrocyte cultures from cerebellum **(A)**, striatum **(B)**, and whole brain **(C)**. Cultures with (△) or without (●) treatment (10 days) with carbamazepine (50 μm). Determinations were performed in triplicate and values given are means of three independent experiments ± SD. **Significantly higher as compared to control cultures ($p < .01$, Student's *t*-test).

apies might also profoundly alter their signal transduction properties.

ACKNOWLEDGMENTS

The authors thank Mrs. Kornelia Adamovic for expert technical assistence and Dr. J. Bauer and K.-N. Klotz for advice and support. This work was supported by the Deutsche Forschungsgemeinschaft grants No. Ca 115/2-5 and Ge 486/9-1.

REFERENCES

Biber K, Walden J, Gebicke-Haerter PJ, Berger M, van Calker D (1996): Carbamazepine inhibits the potentiation by adenosine analogs of agonist-induced inositolphophosphate formation in hippocampal astrocyte cultures. Biol Psychiat 40:563–567

Biber K, Klotz K-N, Berger M, Gebicke-Haerter PJ, van Calker D (1997): Adenosine A_1-receptor-mediated activation of phospholipase C in cultured astrocytes depends on the level of receptor expression. J Neurosci 17:4956–4964

Biden TJ, Browne CL (1993): Cross-talk between muscarinic- and adenosine-receptor signaling in the regulation of cytosolic free Ca2+ and insulin secretion. Biochem J 293:721–728

Chomczynski P, Sacchi N (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159

Costenbader K, Ardaillou N, Marchetti J, Placier S, Ardaillou R (1997): Prostaglandin E2 enhances type 2-bradykinin receptor expression in human glomerular podocytes. Bioch Biophys Acta 1358:142–152

Daval J-L, Deckert J, Weiss SRB, Post RM, Marangos PJ (1989): Upregulation of adenosine A_1-receptors and forskolin binding sites following chronic treatment with caffeine or carbamazepine. Epilepsia 30:26

Dickenson JM, Hill SJ (1994): Interactions between adenosine A_1-receptors and histamine H_1-receptors. Int J Biochem 26:959–969

Durcan MJ, Morgan PF (1990): Prospective roles for adenosine and adenosinergic system in psychiatric disorders. Psychol Med 20:475–486

El-Etr M, Cordier J, Glowinski J, Premont N (1989): A neuroglial cooperativity is required for the potentiation by 2-chloroadenosine of the muscarinic-sensitive phospholipase C in the striatum. J Neurosci 9:1473–1480

Fredholm BB, Abbrachio MP, Brunstock G, Daly JW, Harden KT, Jacobson KA, Leff P, Williams M (1994): VI. Nomen-

clature and classification of purinoceptors. Pharmacol Rev 46:143–156

Freund S, Ungerer M, Lohse MY (1994): A_1 adenosine receptors expressed in CHO cells couple to adenylyl cyclase and to phospholipase C. Naunyn Schmiedebergs Arch Pharmacol 350:49–56

Gebicke-Härter PJ, Bauer J, Schobert A, Northoff H (1989): Lipopolysaccharide free conditions in primary astrocyte cultures allow growth and isolation of microglial cells. J Neurosci 9:183–194

Herlitze S, Garcia DE, Mackie K, Hille B, Scheuer T, Catterall WA (1996): Modulation of Ca2+ channels by G protein beta gamma subunits. Nature 380:258–262

Ikeda SR (1996): Voltage-dependent modulation of N-type calcium channels by G protein beta gamma subunits. Nature 380:255–258

Keshavarzian A, Gordon JH, Wilson C, Urban G, Fields JZ (1992): Chronic ethanol feeding produces a muscarinergic receptor upregulation, but not a muscarinergic supersensitivity in lower esophageal sphincter muscle. J Pharmacol & Exper Therap 260:601–607

Liang BT, Hirsch AJ (1993): Homologous sensitization of embryonic chick atrial myocytes to adenosine: Mediation by adenosine A_1-receptors and guanine nucleotide binding protein. Cardiovasc Res 27:102–110

Ma JY, Catterall WA, Scheuer T (1997): Persistent sodium currents through brain sodium channels induced by G protein beta gamma subunits. Neuron 19:443–452

Mahan LC, McVittie LD, Smyk-Randall EM, Nakata H, Monsma FJ, Gerfen CR, Sibley DR (1991): Cloning and expression of an A_1 adenosine receptor from rat brain. Mol Pharmacol 40:1–7

Manji HK, Potter WZ, Lenox RH (1995): Signal transduction pathways. Molecular targets for Lithium's actions. Arch Gen Psychiat 52:531–543

Miller KJ, Hoffman BJ (1994): Adenosine A_3 receptors regulate serotonin via nitric oxide and cGMP. J Biol Chem 269:27351–27356

Müller S, Lohse MJ (1995): The role of G-protein beta gamma subunits in signal transduction. Biochem Soc Trans 23:141–148

Murthy KS, Makhlouf GM (1995): Adluosine A receptor-mediated activation of phospholipase C beta 3 in intestinal muscle: Dual requirement for alpha and beta subunits of Gi3. Mol Pharmocol 47:1172–1179

Okajima F, Tomura H, Sho K, Akbar M, Madjid MA, Kondo Y (1995): Intracellular cross-talk between thyrotropin receptor and A_1 adenosine receptor in regulation of phospholipase C and adenylate cyclase in COS-7 cells transfected with their receptor genes. Biochem J 306:709–715

Qin N, Platano D, Olcese R, Stefani E, Birnbaumer L (1997): Direct interaction of G beta gamma with a C-terminal G beta gamma-binding domain of the Ca2+ channel α subunit is responsible for channel inhibition by G protein-coupled receptors. Proc Natl Acad Sci 94:8866–8871

Shi D, Nikodijevic O, Jacobson KA, Daly JW (1993): Chronic caffeine alters the density of adenosine, adrenergic, cholinergic, GABA, and serotonin receptors and calcium channels in mouse brain. Cell Mol Neurobiol 13:247–261

Solomon DA, Keitner GI, Miller IW, Shea MT, Keller MB (1995): Course of illness and maintenance treatments for patients with bipolar disorders. J Clin Psychiat 56:5–13

van Calker D, Müller M, Hamprecht B (1978): Adenosine inhibits the accumulation of cyclic AMP in cultured brain cells. Nature 276:839–841

van Calker D, Müller M, Hamprecht B (1979): Adenosine regulates via two different types of receptors the accumulation of cyclic AMP in cultured brain cells. J Neurochem 33:999–1005

van Calker D, Assmann K, Greil W (1987): Stimulation by bradykinin, angiotensin II, and carbachol of the accumulation of inositolphosphates in PC-12 Pheochromocytoma cells: Differential effects of lithium ions on inositol mono- and polyphosphates. J Neurochem 49:1379–1385

van Calker D, Steber R, Klotz K, Greil W (1991): Carbamazepine distinguishes between adenosine receptors that mediate different second messenger responses. Eur J Pharmacol Mol Pharmac Sect 206:285–290

van Calker D, Biber K, Walden J, Gebicke-Härter P, Berger M (1998): Carbamazepine and adenosine receptors. In Manji HK, Bowden C, Belmaker RH (eds), Mechanism of Action of Antibipolar Drugs. Am Psychiat Press, in press

Von Lubitz DKJE, Paul IA, Bartus RT, Jacobson KA (1993a): Effects of chronic administration of adenosine A_1-receptor agonist and antagonist on spatial learning and memory. Eur J Pharmacol 249:271–280

Von Lubitz DKJE, Paul IA, Carter M, Jacobson KA (1993b): Effects of N-cyclopentyl adenosine and 8-cyclopentyl-1,3-dipropylxanthine on N-methyl-D-aspartate induced seizures in mice. Eur J Pharmacol 249:265–270

Von Lubitz DKJE, Lin RC-S, Melman N, Ji X, Carter M, Jacobson KA (1994a): Chronic administration of selective adenosine A_1-receptor agonist or antagonist in cerebral ischemia. Eur J Pharmacol 256:161–167

Von Lubitz DKJE, Paul IA, Ji X, Carter M, Jacobson KA (1994b): Chronic adenosine A_1-receptor agonist and antagonist: Effect on receptor density and N-methyl-d-aspartate induced seizures in mice. Eur J Pharmacol 253:95–99

Wickman KD, Iniguez-Lihui JA, Davenport PA, Taussig R, Krapivinsky GB, Linder ME, Gilman AG, Clapham DE (1994): Recombinant G protein beta gamma subunits activate the muscarinergic-gated atrial potassium channel. Nature 368:255–257

Wiik P (1991): Glucocorticoids upregulate the high affinity receptors for vasoactive intestinal peptide (VIP) on human mononuclear leucocytes in vitro. Regulat Peptides 35:19–30

Williams M (1995): Purinoceptors in central nervous system function. In Bloom FE, Kupfer DJ (eds), Psychopharmacology: The Fourth Generation of Progress. New York, Raven Press, pp 643–655