Modulation of adenylyl cyclase activity in young and adult rat brain cortex. Identification of suramin as a direct inhibitor of adenylyl cyclase

Jiri Stöhr^{a, b}, Jiri Novotny^{a, b *}, Lenka Bourova^{a, b}, Petr Svoboda^{a, b}

^a Department of Biochemistry of Membrane Receptors, Institute of Physiology, Academy of Sciences, Prague, Czech Republic

^b Department of Physiology, Faculty of Natural Sciences, Charles University, Prague, Czech Republic

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Abstract

Adenylyl cyclase (AC) in brain cortex from young (12-day-old) rats exhibits markedly higher activity than in adult (90-day-old) animals. In order to find some possibly different regulatory features of AC in these two age groups, here we modulated AC activity by dithiothreitol (DTT), Fe²⁺, ascorbic acid and suramin. We did not detect any substantial difference between the effects of all these tested agents on AC activity in cerebrocortical membranes from young and adult rats, and the enzyme activity was always about two-fold higher in the former preparations. Nevertheless, several interesting findings have come out of these investigations. Whereas forskolin- and Mn²⁺-stimulated AC activity was significantly enhanced by the addition of DTT, increased concentrations of Fe²⁺ ions or ascorbic acid substantially suppressed the enzyme activity. Lipid peroxidation induced by suitable combinations of DTT/Fe²⁺ or by ascorbic acid did not influence AC activity. We have also observed that PKC- or protein tyrosine kinase-mediated phosphorylation apparently does not play any significant role in different activity of AC determined in cerebrocortical preparations from young and adult rats. Our experiments analysing the presumed modulatory role of suramin revealed that this pharmacologically important drug may act as a direct inhibitor of AC. The enzyme activity was diminished to the same extent by suramin in membranes from both tested age groups. Our present data show that AC is regulated similarly in brain cortex from both young and adult rats, but its overall activity is much lower in adulthood.

Keywords: adenylyl cyclase activity • rat brain cortex • dithiothreitol • peroxidation • suramin

Introduction

Adenylyl cyclase (AC), which catalyses the formation of the ubiquitous second messenger cAMP, plays an important role in neurotransmission in the CNS [1–3]. To date, nine different membrane-bound

mammalian isoforms of AC have been cloned and identified in various tissues [4, 5]. Most of AC isoforms may occur in the CNS, but especially type I, II and V are expressed at high levels in rat brain [6, 7]. The unequal tissue distribution of the individual AC isoforms and diversity of their regulatory features may reflect a specific function of this effector molecule in determining the routing of signals to the cAMP pathway.

* Correspondence to: Dr. J. NOVOTNY,
Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Prague 4, Czech Republic.
Tel.: + 420-241062703; Fax: + 420-241062488
E-mail: novjiri@biomed.cas.cz

The regulation of AC activity by G protein-coupled receptors (GPCRs) is a classically described mechanism in which a stimulatory G protein (G_s) couples neurotransmitters to activation of AC, whereas an inhibitory G protein (G_i) couples to inhibition of AC [8]. This simple scheme, however, does not cover significant diversity in regulation of the individual enzyme isoforms. All AC isoforms are activated by the α subunits ($G_s\alpha$) of G_s protein, but only AC type I, V and VI can be inhibited by direct interaction with the α subunits ($G_i\alpha$) of G_i protein [9]. In addition, the different AC isoforms display very distinct patterns of regulation by the $\beta\gamma$ subunits of G proteins ($G\beta\gamma$) as well as by calmodulin (CaM) and protein kinases [5, 10]. All membrane-bound ACs are inhibited by P-site analogues and, except for type IX, stimulated by the diterpene forskolin [11, 12].

Some previous studies revealed marked alterations in AC activity during postnatal development of rodent brain tissue [7, 13–16]. Whereas AC activity is very low shortly after birth, it consecutively increases to reach a sharp maximum by the end of the second postnatal week. Subsequently, activity of AC rapidly decreases to the adult values, which are comparable to those observed shortly after birth. The same developmental profile of the enzyme activity was observed using different stimulators (forskolin, Mn^{2+} , GTP γ S, fluoride) of AC [7, 13]. Until now, however, no explanation has been provided for the marked differences between AC activity in young and adult brain.

In the present work we have used different modulators (dithiothreitol, ascorbic acid, Fe^{2+} and suramin) in order to analyse in a more detail AC activity in cerebrocortical membrane preparations from young (12-day-old) and adult (90-day-old) rats. The possible effect of phosphorylation on the enzyme activity was also assayed in cerebrocortical homogenates derived from both tested age groups.

Materials and methods

Materials

[α - ^{32}P]ATP and [3H]cAMP were purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Aluminum oxide 90 (neutral, activity I) was from Merck (Darmstadt, Germany). All other chemicals were obtained

from Sigma (St. Louis, MI, USA) and they were of the highest purity available.

Membrane preparation

Young (12-day-old) and adult (90-day-old) male Wistar rats were etherised and killed by decapitation, and their brains quickly removed. Cerebral cortices were rapidly isolated, frozen in liquid nitrogen and stored at $-70^\circ C$. The brain material obtained usually from 6 young or 4 adult animals was always combined together to constitute individual experimental groups. After thawing, the brain tissue was minced with scissors, homogenised in 10 ml of STME buffer per gram wet weight (250mM sucrose, 20mM Tris HCl, 3mM $MgCl_2$, 1mM EDTA, pH 7.6) by using a Potter-Elvehjem homogeniser (Teflon-glass). Unbroken cells and nuclei were removed by low-speed centrifugation (600 x g, 5 min, $4^\circ C$). The supernatant was centrifuged at 200,000 x g for 30 min and the resulting membrane pellet resuspended in STME buffer (total volume corresponded to quintuple initial tissue wet weight). Cerebrocortical membranes were then washed twice in STME buffer using high-speed centrifugation (200,000 x g, 30 min) and, before final freezing in liquid nitrogen, the membrane pellet was resuspended in TMN buffer (20mM Tris HCl, 3mM $MgCl_2$, 150mM NaCl, pH 7.6).

Adenylyl cyclase activity

Activity of adenylyl cyclase was determined according to the method of White [17]. The reaction mixture (total volume of 0.1ml) contained 30 μ g of protein (cerebrocortical membranes), 50mM Tris-HCl buffer (pH 7.4), 5mM $MgCl_2$, 50U/ml pyruvate kinase, 10mM potassium phosphoenolpyruvate, 160 μ g/ml BSA, 0.2 mM 3-isobutyl-1-methylxanthine, 20 μ M GTP, 0.1mM cAMP, 10,000 cpm per sample of [3H]cAMP and 0.4mM ATP with [α - ^{32}P]ATP (about 1×10^6 cpm per sample). Besides forskolin, Mn^{2+} , GTP γ S and AlF_4^- (classical activators of AC activity) some other modulators (dithiothreitol, Fe^{2+} , ascorbic acid, suramin) were also used in concentrations stated concretely in the detailed description of the individual experiments. Before starting the reaction, the assay tubes containing all the components except for the isotope were preincubated for 10 min at $30^\circ C$. The assay was then run for 30 min at $30^\circ C$ and the enzyme reaction terminated by adding 0.2 ml of 0.5 M HCl and heating for 5 min at $100^\circ C$. The cyclic AMP formed was sepa-

rated from other nucleotides and inorganic phosphate by alumina column chromatography [17].

Assay of lipid peroxidation

Lipid peroxidation was measured using the thiobarbituric acid (TBA) test for malondialdehyde (MDA) formation. This assay was conducted according to the previously described protocol [18], with minor modifications. Briefly, after addition of appropriate amounts of different tested compounds (DTT, Fe^{2+} or ascorbic acid), the reaction mixture (100 μl) for AC activity without ATP was incubated for 40 min at 32°C. After this time period, samples were mixed with the same volume (100 μl) of ice-cold 10% trichloroacetic acid and centrifuged at 10,000 rpm for 5 min. The resulting supernatant (150 μl) was mixed with 0.67% TBA (150 μl) and boiled for 10 min. Absorbance was then measured at 535 nm. MDA formation in tested samples was calculated from the calibration curve, which was obtained by parallel measurements of a standard (1,1,3,3-tetramethoxypropane).

Data analysis

All measurements were performed in triplicate in membrane samples prepared by three independent isolation procedures. The numbers represent the average $\pm$ S.E.M. and statistically significant differences within each group were calculated using one-way ANOVA with the Dunnett's multiple comparison post test. When desired, differences between samples from young and adult animals were evaluated by two-way ANOVA followed by the Bonferroni's multiple comparison post test.

Results

Activation of AC by forskolin, $\text{GTP}\gamma\text{S}$, AlF_4^- and Mn^{2+}

Activity of AC was determined in samples of cerebrocortical membranes prepared from young (12-day-old) and adult (90-day-old) rats. First we measured the dose-response curves of forskolin and $\text{GTP}\gamma\text{S}$ to find optimum conditions for using

Fig. 1 Stimulation of AC activity by forskolin (FSK) and $\text{GTP}\gamma\text{S}$. Activity of AC was determined in cerebrocortical membranes from 12-day-old (PD 12) young (open circles) and adult (closed circles) rats and the dose-response curves for forskolin (A) and $\text{GTP}\gamma\text{S}$ (B) were constructed. Data are means ($\pm$ S.E.M.) from a representative experiment assayed in triplicate.

these classical stimulators of AC activity (Fig. 1). Forskolin and $\text{GTP}\gamma\text{S}$ were then used either at maximal stimulating concentrations (5×10^{-5} M and 1×10^{-4} M, respectively) or, in some experiments, at lesser concentrations (1×10^{-5} M and 3×10^{-6} M, respectively) in order to discern possible effects of some other modulators of AC activity. AlF_4^- (10 mM NaF and 1 mM AlCl_3) and Mn^{2+} (10 mM MnCl_2) were used at stated standard concentrations in all experiments. In all conditions, AC activity in cerebrocortical membranes from young rats was markedly (about two-fold) higher than in preparations from adult animals.

Fig. 2 Effect of DTT on AC activity stimulated by forskolin, Mn^{2+} and AlF_4^- . Increasing concentrations of DTT were applied in order to modulate AC activity stimulated by 1×10^{-5} M forskolin (A), 5×10^{-5} M forskolin (B), 1×10^{-2} M Mn^{2+} (C) and AlF_4^- (D) in cerebrocortical membranes from young (open circles) and adult (closed circles) rats. Data are means ($\pm$ S.E.M.) from a representative experiment assayed in triplicate.

Effect of dithiothreitol

According to some earlier observations the thiol-reducing agent dithiothreitol (DTT) can influence activity of AC [19, 20]. In our preliminary experiments we observed that DTT did not affect basal AC activity but it was able to enhance forskolin-stimulated activity of the enzyme (data not shown). This augmenting effect of DTT, however, was detectable only at sub-maximal concentrations of forskolin (compare Fig. 2A and 2B). DTT at its most effective concentration (10 mM) increased forskolin-stimulated AC activity by about 37% and 28% in cerebrocortical membranes from young and adult rats, respectively. When used at 1×10^{-5} M concentration, forskolin in combination with DTT (10 mM) brought about the same stimulatory effect on AC activity as that achieved by maximal stimu-

lating concentration of forskolin (5×10^{-5} M) alone. At lower forskolin concentrations (1×10^{-6} M), the overall maximal stimulatory effect did not manifest, but the activity of AC was significantly increased (by about 20–30%) in the presence of DTT (data not shown). Interestingly, Mn^{2+} -stimulated AC activity was also enhanced by the addition of DTT, similarly as in the case of forskolin (Fig. 2C). The relative increase in AC activity elicited by DTT in cerebrocortical membranes from young rats was higher by about 20% as compared to the increase in samples from adult animals. By contrast, AlF_4^- -stimulated AC activity was rather diminished by DTT (Fig. 2D). Similar decrease (by about 16%) was observed in cerebrocortical membrane preparations both from young and adult rats. Next we analysed the possible effect of DTT on AC activity stimulated by $\text{GTP}\gamma\text{S}$, a non-hydrolyzable analogue of GTP. Interestingly, DTT did not significantly influence AC activity stimulated either by maximal or sub-maximal concentrations of $\text{GTP}\gamma\text{S}$ (Fig. 3).

Effect of Fe^{2+} and ascorbic acid

Some previous studies have demonstrated that free radicals generated by Fe^{2+} /ascorbic acid may alter functioning of the AC signal-transducing system in certain tissues [18, 19, 21, 22]. Our current measurements indicated that the increased concentrations of Fe^{2+} substantially reduce basal as well as forskolin-stimulated AC activity in cerebrocortical membrane preparations both from young and adult rats (Fig. 4). It was shown previously that AC activity in synaptic membrane fraction of rat brain could be potentiated by lipid peroxidation induced by several metal ions (Fe^{2+} inclusive) in the presence of DTT [18, 19]. Surprisingly, here we did not observe any stimulatory effect of any concentration of Fe^{2+} plus DTT (Fig. 5). Results of our concomitant determination of MDA formation demonstrated that Fe^{2+} in the range of micromolar concentrations in the presence of increased levels of DTT (over 1 mM) evidently caused lipid peroxidation (Fig. 6), but activity of AC was not changed under these conditions. Interestingly enough, Fe^{2+} ions at a concentration of 1×10^{-4} M induced a quite perceptible increase in MDA formation, which was totally abolished by the addition of 5mM DTT (Fig. 6A). In addition, DTT markedly attenuated the negative

Fig. 3 Effect of DTT on AC activity stimulated by GTP γ S. The effect of DTT (1×10^{-2} M) on AC activity stimulated by a maximally effective concentration of GTP γ S (1×10^{-4} M) was tested in cerebrocortical membranes from young (PD 12) and adult (PD 90) rats (A). Increasing concentrations of DTT were used in order to modulate AC activity stimulated by a sub-maximal concentration of GTP γ S (3×10^{-6} M) in cerebrocortical membranes from young (open circles) and adult (closed circles) rats. Data are means ($\pm$ S.E.M.) from a representative experiment assayed in triplicate.

Fig. 4 Effect of Fe²⁺ on basal and forskolin-stimulated AC activity. Increasing concentrations of Fe²⁺ (FeSO₄) were applied in order to modulate basal (A) and forskolin-stimulated (B) activity of AC in cerebrocortical membranes from young (open circles) and adult (closed circles) rats. Data are means ($\pm$ S.E.M.) from a representative experiment assayed in triplicate.

effect of increased Fe²⁺ concentrations on AC activity. While 0.1mM Fe²⁺ reduced basal AC activity

by about 67%, the enzyme activity dropped only by 16% in the presence of 5 mM DTT (compare Fig. 4

Fig. 5 Effect of Fe^{2+} /DTT on basal AC activity. Activity of AC in cerebrocortical membranes from young (open circles) and adult (closed circles) rats was measured in the presence of 5×10^{-3} M DTT and increasing concentrations of Fe^{2+} (FeSO_4). Data are means ($\pm$ S.E.M.) from a representative experiment assayed in triplicate.

and 5). In the following experiments we evaluated the presumed role of ascorbic acid in modulation of

AC activity. In spite of our expectations, ascorbic acid at lower concentrations (up to 1mM) did not influence either basal or forskolin-stimulated AC activity and at high concentrations it strongly diminished the enzyme activity in membrane preparations from both young and adult rat brain cortex (Fig. 7). Ascorbic acid at optimal concentration (about 1×10^{-5} – 1×10^{-4} M) was equally effective in eliciting lipid peroxidation in both types of tested samples (Fig. 6C).

Effect of suramin

Suramin, a polysulphonated naphthylurea, can presumably exert many different biological actions. Besides others, suramin is known to act directly on G protein α subunits and to block their activation by inhibiting the exchange of GDP for GTP [23–25]. In the present study we decided to test this potent G protein antagonist for its possible modulatory effect on AC activity. Quite unexpectedly, suramin exercised a clear inhibitory effect on basal as well as on differently stimulated activity of AC and higher concentrations (above 1×10^{-4} M) of this compound totally diminished the enzyme activity (Fig. 8). The pronounced negative effect was manifested to the same extent in cerebrocortical membranes both from young and adult rats. Since the

Fig. 6 Effect of Fe^{2+} /DTT and ascorbic acid on lipid peroxidation. Lipid peroxidation in cerebrocortical membranes from young (circles) and adult (triangles) rats was determined as MDA formation using either increasing concentrations of Fe^{2+} (FeSO_4) in the absence (open symbols) or presence (closed symbols) of 5×10^{-3} M DTT (A) or increasing concentrations of DTT in the absence (open symbols) or presence (closed symbols) of 1×10^{-5} M Fe^{2+} (B) or in the presence (open symbols) of increasing concentrations of ascorbic acid (C). Data are means ($\pm$ S.E.M.) from a representative experiment assayed in triplicate.

Fig. 7 Effect of ascorbic acid on basal and forskolin-stimulated AC activity. Basal (A) and forskolin-stimulated (B) activity of AC in cerebrocortical membranes from young (open circles) and adult (closed circles) rats was measured in the presence of increasing concentrations ascorbic acid. Data are means ($\pm$ S.E.M.) from a representative experiment assayed in triplicate.

IC₅₀ of suramin (about 3×10^{-5} M) was practically the same for basal and directly (Mn^{2+}) stimulated AC activity as well as for the enzyme activity stimulated through the G proteins (AlF_4^-) or both directly and through the G proteins (forskolin), suramin might be considered to act as a direct inhibitor of the catalytic subunits of AC. This assumption was confirmed by subsequent experiments with plasma membranes derived from S49 cyc- lymphoma cells, which lack $\text{G}_s\alpha$ protein [26]. Fig. 9 shows that suramin (1×10^{-4} M) substantially reduced AC activ-

ity in cyc- membranes stimulated either by Mn^{2+} or forskolin to the values corresponding to basal enzyme activity, which come close to zero.

Effect of phosphorylation

In order to find possible differences between the regulation of AC activity in cerebrocortical preparations from young and adult rats, finally we evaluated the potential modulatory role of phosphorylation. It is known that some types of AC from higher organisms can be regulated by protein kinases [5, 27, 28]. Posttranslational modification of AC by phosphorylation may either stimulate or inhibit the enzyme activity. Therefore it was of interest to assess the potential effect of AC phosphorylation catalysed by protein kinase C (PKC) as well as by protein tyrosine kinases (PTKs). For this purpose, phorbol myristate acetate (PMA) and pervanadate (PV) were employed as effective activators of PKC and PTKs, respectively [29, 30]. We determined basal as well as forskolin- and AlF_4^- -stimulated AC activity in cerebrocortical homogenates prepared from both young and adult rats. All tested samples were either untreated (control) or treated with PMA or PV in order to induce the requisite type of phosphorylation. As seen in Fig. 10, treatment of cerebrocortical homogenates with protein kinase activators apparently did not influence AC activity, because it was not significantly altered in any of the used experimental conditions.

Discussion

In the present work we sought to compare more closely AC activity in cerebrocortical membranes from young (12-day-old) and adult (90-day-old) rats. These two age intervals were chosen because some earlier studies demonstrated that activity of AC dramatically changes during postnatal development of rodent brain and, as a rule, this enzyme activity reaches a sharp maximum around PD 12 and then quickly declines to the adult values [7, 13, 15].

Our current results indicated that AC activity was always considerably (about two-fold) higher in cerebrocortical preparations derived from young than from adult animals. Nevertheless,

Fig. 8 Effect of suramin on AC activity in cerebrocortical membranes. Increasing concentrations of suramin were applied in order to modulate basal (A) as well as AC activity stimulated by $1 \times 10^{-5} \text{ M}$ forskolin (A), $1 \times 10^{-2} \text{ M}$ Mn^{2+} (B) and AlF_4^- (C) and $5 \times 10^{-5} \text{ M}$ forskolin (D) in membrane preparations from young (open circles) and adult (closed circles) rats. Data are means ($\pm$ S.E.M.) from a representative experiment assayed in triplicate.

some interesting differences could have been observed between the ability of various activators to stimulate the enzyme activity in both types of tested samples, especially when it was modulated by the addition of dithiothreitol (DTT) to the reaction mixture. Whereas DTT alone did not significantly influence basal activity of AC, the enzyme activity stimulated by forskolin or Mn^{2+} was markedly enhanced by high concentrations (over 1 mM) of this agent. The potentiation effect of DTT was detectable only when using sub-maximal stimulating concentrations of forskolin; DTT was not able to further enhance AC activity stimulated by maximally effective forskolin doses. DTT was a bit more effective in exercising these positive effects on AC activity in samples from young than adult rats. Interestingly, DTT did not influence AC activity stimulated by GTP γ S and somewhat reduced AlF_4^- -stimulated AC activity in samples from both tested age groups. These results indicate that DTT can enhance activity of AC, which is stimulated directly (by forskolin or Mn^{2+}). By contrast, the lack of clear effect of DTT on AC activity stimulated through the G proteins (Gs) suggests that this compound apparently does not strongly influence functioning of these regulatory proteins and their interaction with AC. Our finding of no direct effect of DTT on basal AC activity is in line with some previously published observations [19, 31]. On the other hand, Ozawa and colleagues detected a certain increase

Fig. 9 Effect of suramin on AC activity in cyc- membranes. Basal, Mn^{2+} ($1 \times 10^{-2} \text{ M}$) - and forskolin ($1 \times 10^{-5} \text{ M}$)-stimulated AC activity in plasma membranes derived from S49 cyc- lymphoma cells was determined in the absence (empty bars) or presence (solid bars) of $1 \times 10^{-4} \text{ M}$ sodium suramin. Data are means ($\pm$ S.E.M.) from a representative experiment assayed in triplicate.

Fig. 10. Effect of phosphorylation on AC activity. Phosphorylation in cerebrocortical homogenates from young (empty bars) and adult (solid bars) rats was catalysed either by protein kinase C (PKC) or protein tyrosine kinases (TRKs). PKC was activated by preincubation of samples with 1 μ M phorbol myristate acetate (PMA) for 10 min at 30°C [57]. Preincubation (10 min at 30°C) of samples with 10 μ M pervanadate (PV) was used for activation of TRKs [58]. Basal, forskolin (1 $\times$ 10⁻⁵ M)- and AIF₄⁻-activated AC cyclase was then determined as described in Methods. Data are means ($\pm$ S.E.M.) from a representative experiment assayed in triplicate.

in basal AC activity after pretreatment of thyroid plasma membranes with DTT [32]. The positive modulatory role of DTT on AC activity stimulated by forskolin or Mn²⁺ has not been previously noted. It has been shown, however, that DTT may partially prevent inactivation of AC by some P-site inhibitors [33]. Likewise, Lin and colleagues observed that inhibition of the enzyme activity caused by low concentrations ($\leq$ 0.1 mM) of organic mercurials could be reversed by the addition of DTT [34]. This is in accord with our present finding that DTT was able to reduce appreciably the negative effect of increased Fe²⁺ concentrations on AC activity. These results suggest that protection of -SH groups of the enzyme from oxidative reactions might be of great importance for proper AC functioning.

Next we examined the potential effects of various concentrations of Fe²⁺ ions on basal and forskolin-stimulated AC activity. Increased levels of Fe²⁺ (over 10 μ M) diminished AC activity simi-

larly in cerebrocortical membranes from both young and adult animals. As mentioned above, this negative effect was strongly attenuated in the presence of DTT. However, neither combination of Fe²⁺ with DTT had stimulating effect on the enzyme activity, despite lipid peroxidation took place under some tested conditions. Our present results thus do not show any correlation between activity of AC and ambient levels of lipid peroxides. This is a bit surprising, since Baba and co-workers reported earlier that AC in synaptic membrane fraction of rat brain was activated selectively by stimulation of peroxidation [18, 19]. A short transient increase in AC activity was also elicited by lipid peroxidation in sarcolemmal membranes, which was followed by depression of the enzyme activity [22]. In our experimental conditions, however, AC activity was not affected even by lipid peroxidation induced by optimal concentrations of ascorbic acid. By contrast, higher concentrations of ascorbic acid substantially reduced the enzyme activity. All these

observations are in line with data published by [35, 36], who did not find any stimulation of AC in neuroblastoma cells exposed to Fe^{2+} /DTT. Moreover, lipid peroxidation was associated with reduction of AC activity [35]. Likewise, Shin and co-workers have recently reported that AC activity is attenuated by aldehydic products of lipid peroxidation in GH_4C_1 cells [37]. These observations conform to the notion about generally deleterious effects of reactive O_2 species and peroxidation on transmembrane signalling.

In the following set of experiments we wished to test the possibility that the higher AC activity determined in brain cortex preparations from young compared to adult rats could be perhaps somehow related to the enzyme interaction with the G proteins. Therefore we decided to take advantage of using suramin as a known blocker of the G protein function (inhibition of the GDP/GTP exchange) and tried to test this compound for its presumed modulatory effect on AC activity. Rather surprisingly, suramin inhibited not only AC activity stimulated through the G proteins but it was also highly effective in diminishing the basal as well as forskolin- or Mn^{2+} -stimulated enzyme activity. Similar inhibition was found in cerebrocortical preparations from both tested age groups. These observations indicated that suramin may function as a direct inhibitor of the catalytic subunit of AC. Further evidence in favour of this assumption was brought by the assessment of suramin effect on AC activity in plasma membranes derived from S49 cyc⁻ cells. The addition of suramin to these membranes, which lack $\text{G}_s\alpha$ protein [26], completely abolished the stimulating effect of Mn^{2+} and forskolin on AC activity.

Besides showing a high potential of suramin in blocking the G protein function [23–25], a number of previous studies demonstrated its ability to impede binding of several growth factors to their cellular receptors [38–40]. Moreover, suramin has also been identified as an agent strongly interfering with the signal transduction pathways by inhibiting protein kinase C (PKC) and phosphoinositide synthesis [41–43]. Nevertheless, there is only little information available about the potential relationship between suramin and AC. It has been observed earlier that suramin as a P_2 -purinoceptor antagonist could markedly reduce ATP- or UTP-mediated inhibition of forskolin-stimulated cAMP accumulation in cultured mesangial cells [44]. Similarly, suramin

in a concentration-dependent manner attenuated ADP-induced inhibition of PGE_1 -stimulated cAMP accumulation in human platelets [45]. Suramin alone, however, had no significant effect on the basal levels of intracellular cAMP and it was able to inhibit accumulation of cAMP induced by sub-maximal concentrations of PDE_1 [45]. Interestingly enough, Lopez-Lopez and co-workers reported that treatment of the breast cancer cell line MCF-7 with suramin resulted in rapid and significant enhancement of AC activity (increase in intracellular cAMP levels), but no such effect was observed in the prostate cancer cell line PC3 [46]. Results of many previous experiments have thus disclosed suramin as an agent causing different perturbations in cellular signalling, including variable effects on the cAMP metabolism. Our present data bring a new evidence about direct interaction of suramin with AC and suggest that this drug should be considered not only as a potent inhibitor of G proteins and PKC, but of AC activity as well.

As a last step toward investigating the basis for markedly different activity of AC in brain cortex from young and adult rats, we assessed the possible modulatory effect of phosphorylation. Basal as well as forskolin- and AlF_4^- -stimulated AC activity was determined in cerebrocortical homogenates prepared from both age groups. Before measurement of AC activity, the samples were either untreated (control) or treated with phorbol myristate acetate or pervanadate in order to induce phosphorylation catalysed by PKC or protein tyrosine kinases (PTKs), respectively [29, 30]. However, we did not find any significant difference in AC activity in any tested samples after activation of these protein kinases. It has been shown previously that posttranslational modification of some types of AC by phosphorylation may either stimulate or inhibit the enzyme activity [5, 27, 28]. Whereas AC II and AC VII can be stimulated by PKC [47, 48], PKC-mediated phosphorylation of AC IV and AC VI seems to be inhibitory [49, 50]. Some *in vitro* experiments indicated that PKC may enhance activity of AC V [51]. However, treatment of cells with phorbol esters did not alter AC V activity to any significant extent and it remains to be determined whether or not is activity of this type of AC altered by PKC in intact cells [52]. Most recently, it has been noted that AC activity is also regulated by the activation of both receptor- and nonrecep-

tor-linked tyrosine kinases and both stimulatory and inhibitory effects mediated by PTKs have been described [27, 30, 53, 54]. As mentioned above, treatment of cerebrocortical homogenates with any used protein kinase activator did not significantly influence AC activity in our experimental conditions. It might be therefore assumed that the potentially variable effects of phosphorylation are more or less balanced at the level of an overall cerebrocortical activity of AC.

In conclusion, our present observations indicate that AC is regulated similarly in brain cortex from young (12-day-old) and adult (90-day-old) rats, but the enzyme activity is much lower in adult than in young animals. As argued previously, the difference between AC activity in these two age groups is not explicable on the basis of the developmental expression profiles of either AC I, II, IV and VI or different G proteins [13, 55]. It might be speculated, however, that the complement of some other types of AC could be changed in adulthood. This supposition is supported by our recent finding of altered characteristics of [³H]forskolin binding in cerebrocortical membranes from adult compared to young rats [56]. Nevertheless, a possibility can not be ruled out that AC activity in adult rat brain cortex might be perhaps affected by some yet not known negative regulatory factor/mechanism, which is switched on shortly after maturation.

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