Circadian rhythm in the Ca^{2+}-inhibitable adenylyl cyclase activity of the rat striatum

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Abstract In the present study, we demonstrate that the Ca^{2+}-inhibitable adenylyl cyclase (AC) activity in the striatum exhibits a daily oscillation with a peak occurring around 10:00 h. A circadian fluctuation of the AC activity evoked by an A2a adenosine-selective agonist was also observed. Intrastriatal injection of an A2a-selective adenosine agonist or antagonist during the interval in which the Ca^{2+}-inhibitable AC activity was at its peak resulted in a more significant alteration of locomotor activity than those observed at a later interval. The marked circadian variation in the Ca^{2+}-inhibitable AC activity in the striatum appears to cause a circadian fluctuation in the action of at least one neuromodulator.

Key words: Adenylyl cyclase; Adenosine; Striatum; Circadian rhythm; Locomotor activity

1. Introduction

Genes of at least 10 distinct mammalian cyclases (ACs), which can be further divided into five subfamilies, have been reported [1]. Although these enzymes can all be activated by the α subunit of Gs proteins, each is under very distinct regulation. For example, some ACs (AC5 and AC6) can be inhibited by Ca^{2+}, while others (AC1, AC3 and AC8) are activated by Ca^{2+} in the presence of calmodulin [2]. In addition, a recently cloned AC subtype, AC10, was found to be inhibited by Ca^{2+}/calmodulin-activated protein phosphatase, calcineurin [3]. This heterogeneous multigene AC family thus appears to play a very critical role in the integration of multiple signals, especially those occurring in the nervous system. Nevertheless, regulation of these ACs under physiological conditions remain largely uncharacterized. In the present study, we have demonstrated a circadian variation in the Ca^{2+}-inhibitable adenylyl cyclase (AC) activity in the rat striatum. In addition, this circadian regulation of the Ca^{2+}-inhibitable AC activity subsequently leads to a circadian fluctuation of the cAMP signaling evoked by one AC-coupled neuromodulator, adenosine, in the striatum.

2. Materials and methods

2.1. Materials

Adenosine, 8-(3-chlorostyryl)caffeine, and CGS21680 were obtained from Research Biochemicals Int. (Natick, MA, USA). Forskolin and cAMP were obtained from Sigma (MO, USA).

2.2. Adenylyl cyclase assay

Adenylyl cyclase activity was assayed as described previously [4] with slight modification. In brief, the striatum tissues were removed, and sonicated in ice-chilled lysis buffer (10 mM EDTA, 20 mM Tris, 250 mM sucrose, 1 mM PMSF amd 40 μM leupeptin, pH 7.4) using an ultrasonic cell disrupter (40% output power) for a total of 45 seconds. The homogenate was centrifuged at $40000 \times g$ for 30 min and the membrane fractions and cytosolic fractions collected. Adenylyl cyclase activity assay was performed at 37°C for 10 min in a 400-μl reaction mixture containing 1 mM ATP, 100 mM NaCl, 50 mM HEPES, 6 mM $MnCl_2$, 1 μM GTP, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) and 20 μg membrane protein. Reactions were stopped by 0.6 ml 10% TCA. The cAMP formed was isolated by Dowex chromatography (Sigma) and assayed by radioimmunoassay as described [4]. No significant difference was found by adding 20 mM creatine phosphate (Sigma), and 100 U/ml creatine phosphokinase (Sigma) to the cyclase reaction. The enzyme activity was linear up to 20 min with protein up to 100 μg. All samples were assayed in triplicate.

2.3. Animals and Drug administration in striatum

Male Sprague-Dawley rats (~250 g) were obtained from the Institute of Biomedical Sciences/Academia Sinica. Animals were housed three per cage under a 12-h light/dark cycle (light on at 6:30 h). Rats were subject to stereotaxic surgery. Twenty-three-gauge stainless steel cannulae were implanted bilaterally into the striatum at the following coordinates: AP, ±0.4 mm from bregma; ML, ±2.7 mm from midline; and DV, −5.4 mm below the skull surface). One week following surgery, the desired reagent was injected into the striatum through a 30-gauge injection needle connected to a 10 μl Hamilton microsyringe by 0.5 m polythylene tubing. Drug solutions were introduced into the PE tubing and delivered into the striatum through the injection needle manually at a rate of 0.5 μl/min. A volume of 1.5 μl was injected into each striatum for all animals. Two minutes subsequent to each injection, locomotor activity was measured for 10 min as described elsewhere [5]. Briefly, animals were placed in an activity monitor (Coulbourn Instrument, PA, USA) equipped with 16×16 horizontal sensors. These sensors were used to localize the animal's floor position. Locomotor activity was measured by total number of beam breaks in an X–Y plane recorded every 10 ms.

3. Results and discussion

To determine whether the Ca^{2+}-inhibitable AC activity in the rat striatum exhibited a circadian fluctuation, we removed striatum tissues at 4-h intervals across a 24-h period, and determined the AC activity. As shown in Fig. 1, the Ca^{2+}-inhibitable AC activity exhibited a daily oscillation with a peak occurring around 10:00 h. The adenylyl cyclase activity evoked by forskolin (5 μM) in the absence or presence of calcium (30 μM) at 2:00 h is 1397 ± 475 pmole/mg per min and 84 ± 32 pmole/mg per min respectively (12 determinants from 4 animals), suggesting that more than 90% of the forskolin-evoked AC activity in the striatum is Ca^{2+}-inhibitable under our experimental conditions. Therefore, the Ca^{2+}-inhibitable AC must play an important role in the striatum. Our results are consistent with the notion that expression of Ca^{2+}/

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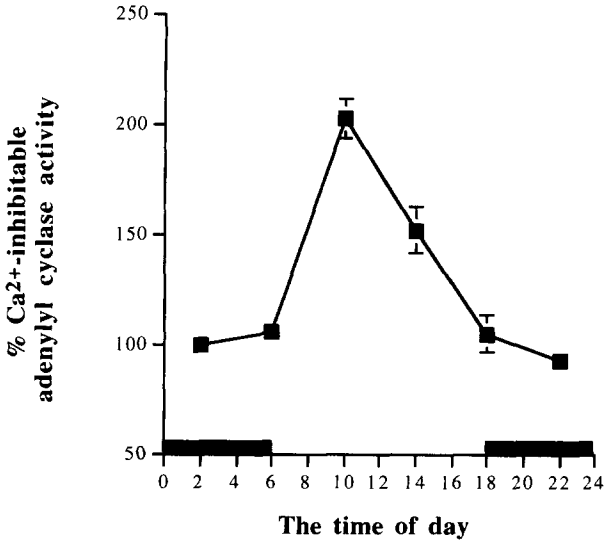

Fig. 1. Circadian regulation of the Ca^{2+}-inhibitable AC activity. Membrane fractions were collected from the striatum, washed twice in an EGTA buffer to remove the endogenous calcium and resuspended in a EGTA/calcium buffer to control the free calcium concentration at zero or 30 μM for adenylyl cyclase assay. The Ca^{2+}-inhibitable AC activities in response to forskolin (5 μM) at the indicated time of day were then determined. Values represent the mean ± S.E.M. of 12 determinations from 4 animals, and are expressed as percentages of the Ca^{2+}-inhibitable AC activity (1397 ± 475 pmole/mg per min) at 2:00 h. The closed bars represent the dark phase.

calmodulin-stimulated AC activity in the striatum is relatively low [6]. Such circadian regulation of the Ca^{2+}-inhibitable AC activity appears to be region-specific. For example, no significant circadian variation in the Ca^{2+}-inhibitable AC activity was observed in the hippocampus (data not shown).

To reduce the possible influence of Gsα on AC activity [7], we substituted GDPβS and $MnCl_2$ for GTP and $MgCl_2$ respectively in our AC assay (Fig. 2). Although GDPβS/$MnCl_2$ markedly reduced the Ca^{2+}-inhibitable AC activity, both total AC activity and the Ca^{2+}-inhibitable AC activity at 10:00 h in the striatum remained significantly higher than those at 2:00 h (Fig. 2). In addition, no significant change in the Gsα protein levels was detected throughout the 24-h period (n = 2, data not shown). These results suggest that regulation of the effector enzyme itself, but not Gsα protein, may lead to the circadian variation of the Ca^{2+}-inhibitable AC activity.

At present, two Ca^{2+}-inhibitable ACs (AC5 and AC6) have been identified [8]. Both Acs are inhibited by physiologically relevant, low micromolar concentrations of Ca^{2+}, and have been reported to exist in the brain [8,9]. Using in situ hybridization analysis, Mons and Cooper [10] demonstrated that AC5 mRNA exhibits a highly selective distribution in the striatum, while AC6 mRNA exists ubiquitously at relatively very low levels throughout the rat brain. In addition, AC5 transcripts were found to coexist with D1-dopamine receptor and M1-muscarinic receptor in medium-sized striatal neurons [10], suggesting a potentially significant role of AC5 in integrating dopaminergic and muscarinic signals. Although the transcript levels of AC5 are significantly higher than those of AC6 in the striatum, the regional expressions of the functional AC5 and AC6 proteins are currently unknown due to the lack of highly specific sub-type antisera. Therefore it cannot be stated which Ca^{2+}-inhibitable AC is under circidian regulation in the striatum.

Recent studies suggest that each isoform of AC is expressed distinctly in specific regions of the brain [11]. The specific tissue distribution of these different ACs supports biochemical evidence for distinct modes of regulation of cAMP levels [1]. Regulation of the AC family has thus aroused a great deal of interest in the past few years. Recently, protein phosphorylation has been implicated in the modulation of AC activities. For example, AC6 has been reported to be inhibited by protein kinase A (PKA) in a feedback inhibition manner [12]. In PC12 cells, we have previously reported that phosphorylation of AC6 may account for its inhibition during desensitization of A2a adenosine receptor (A2a-R)-mediated cAMP response [13]. Most intriguingly, activity of AC5 was found to be upregulated by PKC-mediated phosphorylation, but inhibited by PKA-mediated phosphorylation [14,15]. Since circadian regulation of protein kinase activities and protein phosphorylation have been shown in several different tissues [16,17], phosphorylation thus has become one of the most plausible molecular mechanisms underlying the circadian rhythm of the Ca^{2+}-inhibitable AC activity.

Rapid changes in receptor function over 24 h in the central nervous system have been reported for many different neurotransmitters [18]. In addition to circadian fluctuation of receptor activities, our finding suggests that circadian regulation of the Ca^{2+}-inhibitable AC activity may also produce circadian fluctuations of receptor-mediated cAMP levels. To test the above hypothesis, we have examined the AC activity evoked by an AC-coupled A2a-R. As demonstrated in Fig. 3, AC activity evoked by an A2a-selective agonist, CGS21680, also

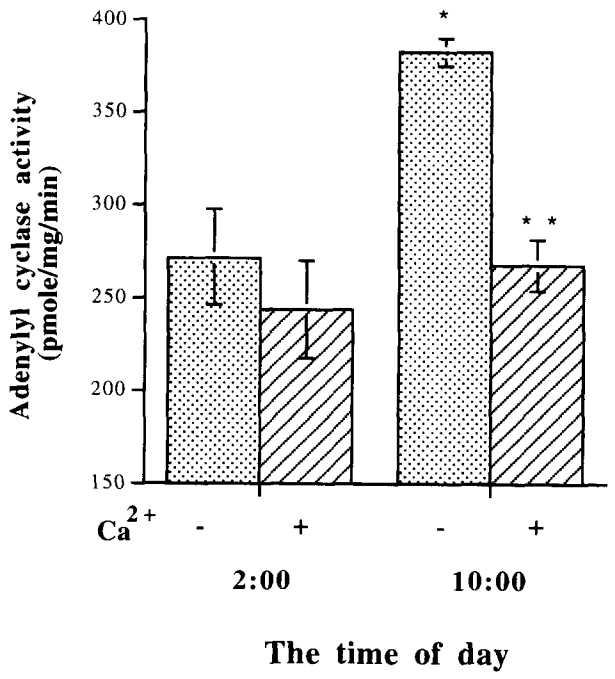

Fig. 2. Circadian regulation of the Ca^{2+}-inhibitable AC activity was largely independent of a change in Gsα activity. AC activities were measured in the presence of GDPβS (500 μM) and $MnCl_2$ (10 mM) as described in section 2. Values represent the mean ± S.E.M. of at least 5 determinations from 2 animals. Statistical significance: $*P < 0.05$ compared to the FK-evoked AC activity at 2:00 h, $**P < 0.01$ compared to the FK-evoked AC activity in the absence of Ca^{2+} at 10:00 h (two-tailed Student's test).

exhibited a circadian fluctuation which peaked around 10:00 h. Again the Ca^{2+}-inhibitable AC activity constituted at least 90% of the A2a-R-stimulated AC activity under our experimental conditions. This observation supports the hypothesis that the circadian regulation of the effector enzyme itself may produce a daily oscillation of the receptor's signal.

To examine whether the circular variation of A2a-R-induced cAMP response in the striatum has any physological significance, an A2a-selective agonist (CGS21680) or an antagonist [8-(3-chlorostyryl)caffeine] [19] was injected intrastriatally. Since one of the striatal functions is control of movement, we determined the locomotor activity of these drug-treated animals. Interestingly, an inverse phase pattern was observed between the activity and the cAMP contents (Fig. 4). CGS21680 (CGS), which resulted in an elevation of cellular cAMP content, significantly reduced locomotor activity in rats at the interval (9:30–10:30 h) in which the Ca^{2+}-inhibitable AC activity was at its peak, but not at a later interval (16:30–17:30 h) when the Ca^{2+}-inhibitable AC activity was relatively low. Injection of the A2a-selective antagonist (CSC) markedly enhanced locomotor activity at both time intervals tested, suggesting that a portion of the striatal A2a-R was active under physiological conditions. Again, the effect of CSC is more significant around 10:00 h than that observed around 17:00 h. These results suggest that circadian regulation of the Ca^{2+}-inhibitable AC activity might contribute to the circadian oscillation of the physiological functions of a neuromodulator.

Various lines of evidence suggest a powerful antagonistic A2a (adenosine)-D2 (dopamine) interaction in the ventral striatal system [20]. Therefore, A2a-selective agonists or antagonists might be useful in the treatment of movement disorders caused by a dysfunction of the striatal dopaminergic

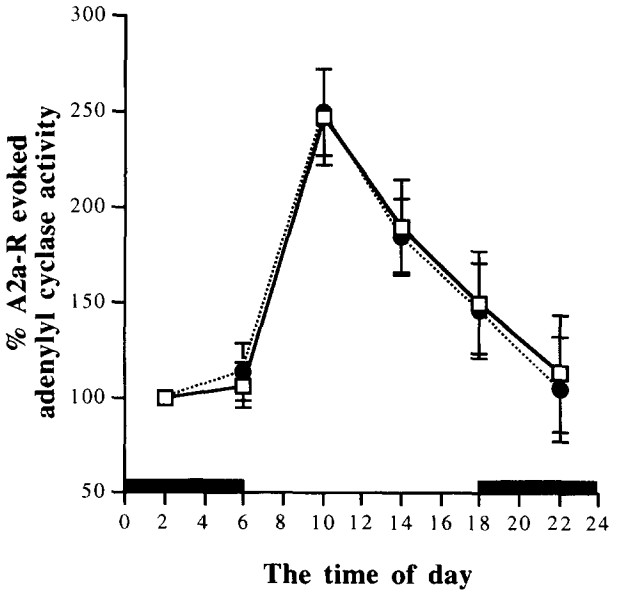

Fig. 3. Circadian regulation of the A2a-R-evoked AC activity. Membrane fractions from the striatum were obtained as described in Fig. 1. The total AC (open squares) and the Ca^{2+}-inhibitable AC activities (closed circles) in response to CGS21680 (100 μM) at the indicated time of day were then determined. Values represent the mean ± S.E.M. of 12 determinations from 4 animals, and are expressed as percentages of the total AC activity (194 ± 60 pmole/mg per min) and the Ca^{2+}-inhibitable AC activity (171 ± 46 pmole/mg per min) at 2:00 h.

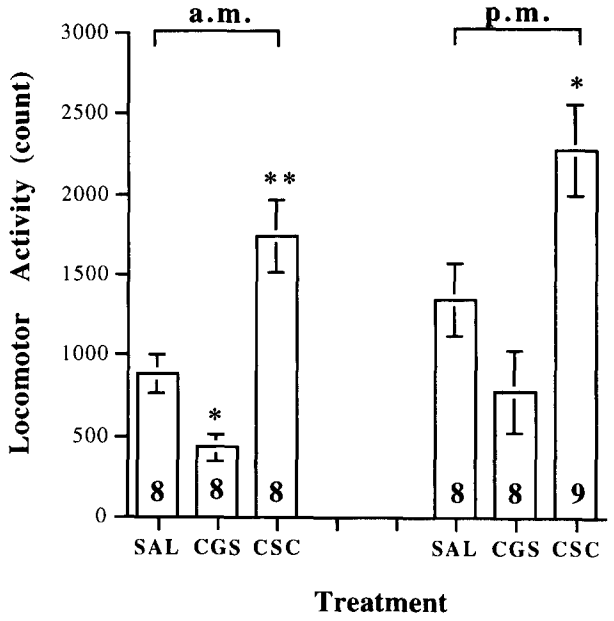

Fig. 4. Effect of an adenosine agonist (CGS21680) and an antagonist [8-(3-chlorostyryl)caffeine] on locomotor activity. Animals were intrastriatally injected with 1.5 μl of the indicated reagent [SAL, 2% DMSO in saline; CGS, CGS21680, 1 μg/μl; CSC, 8-(3-chlorostyryl)-caffeine, 0.02 μg/μl] bilaterally in the morning (9:30–10:30 h) or afternoon (16:30–17.30 h). Two minutes after the injection, locomotor activities were measured for 10 min as described in section 2. Statistical significance: differences between drug-treated animals and vehicle-injected control animals, $*P < 0.05$ and $**P < 0.01$, respectively; ANOVA test.

neurotransmission, such as Parkinson's disease and Huntington's disease [21]. Our observations suggest that adenosine agonists and antagonists appear to have a better manipulation of locomotor activity when the Ca^{2+}-inhibitable AC activity is at its peak, and therefore may lead to a greater therapeutic application of adenosine in the treatment of movement disorders.

Anholt [22] has proposed an intriguing and attractive hypothesis that ACs might serve as 'molecular coincidence detectors' in the nervous system to integrate and to screen for 'meaningful signals' from noise. In the present study, we report that the Ca^{2+}-inhibitable AC activity in the striatum exhibited a circadian fluctuation with one clear peak occurring at 10:00 h. Such significant variation in the Ca^{2+}-inhibitable AC activity throughout the day led to a circadian fluctuation in the action of at least one neuromodulator, and may effectively contribute to the well-known circadian rhythms of susceptibility to many central nervous system drugs.

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