Effective Inhibition by Pentobarbital of Forskolin-Stimulated Adenylate Cyclase Activity in Rat Brain

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The effect of pentobarbital on the adenylate cyclase system was examined in synaptosomal membranes from rat brain. Pentobarbital inhibited forskolin-stimulated enzyme activity more effectively than the basal and Mn^{2+}-stimulated enzyme activities. The degree of inhibition of the enzyme activity by pentobarbital was increased by the presence of forskolin in a concentration-dependent manner. No significant difference is observed in the degree of the inhibition by pentobarbital between the basal and forskolin-stimulated activities in the membranes prepared from the peripheral tissues.

Keywords adenylate cyclase; pentobarbital; forskolin; rat brain

Forskolin, a diterpene from the root of *Coleus forskolii*, activates adenylate cyclase,[1] and increases the intracellular level of cyclic adenosine monophosphate (cyclic AMP).[2] It has been proposed that the diterpene acts directly on the catalytic unit of the adenylate cyclase system[2] and also influences the interaction between G_s (guanine nucleotide-binding stimulatory regulatory protein) and the catalytic unit.[3-5]

On the other hand, the cyclic AMP level in the cerebrospinal fluid of epileptic patients is generally higher than that in normal subjects.[6] High levels of this nucleotide are observed in the brain after the admistration of convulsants or electroshock, and the increase is suppressed by anticonvulsants.[7-9] Additionally, the intraventricular injection of dibutyryl cyclic AMP is epileptogenic in experimental animals.[10] We have studied the effect of barbiturates on the adenylate cyclase system in the central nervous system. Recently, we have indicated that barbiturates primarily suppressed the activation of the catalytic unit through the coupling with G_s.[11,12]

In this study, we attempted to examine the effect of pentobarbital on adenylate cyclase activity in the forskolin-stimulated state in rat brain synaptosomal membranes. We report here that pentobarbital more effectively inhibits forskolin-stimulated enzyme activity than that in the absence of forskolin, and that the degree of the inhibition by pentobarbital is increased by the presence of forskolin in a concentration-dependent manner. But, no significant difference was observed in the degree of inhibition by pentobarbital of these enzyme activities in the peripheral tissues.

Experimental

[2-^{3}H] adenosine triphosphate (ATP) was purchased from Amersham International Ltd. All other drugs and chemicals were of reagent grade from standard commercial sources.

Synaptosomal membranes were prepared from brains of male Wistar rats (150—200 g). The brains were homogenized in 10 volumes of 320 mM sucrose solution containing 5 mM Tris–HCl and 1 mM ethylene diaminetetraacetic acid (EDTA) (pH 7.4) with a Potter-Elvehjem homogenizer. The homogenate was centrifuged at $800 \times g$ for 10 min to remove the cell nuclei and debris. The post-nuclear supernatant was centrifuged at $10000 \times g$ for 30 min. The pellet was resuspended in 20 volumes of 5 mM Tris–HCl buffer (pH 7.4). After standing for 30 min, the suspension was centifuged at $20000 \times g$ for 30 min. The resulting pellet was suspended in 50 mM Tris–HCl buffer (pH 7.4) containing 250 mM sucrose. To prepare the plasma membranes from peripheral tissues, the post-nuclear supernatant from hearts and kidneys of male Wistar rats (150—200 g) was centrifuged at $100000 \times g$ for 30 min. The resultant pellet was suspended in 50 mM Tris–HCl buffer (pH 7.4) containing 250 mM sucrose.

Adenylate cyclase activity was measured according to the method of Salomon *et al.*[13] with some modifications.[14] Protein was determined by the method of Lowry *et al.*[15] using bovine serum albumin as the standard.

Results and Discussion

Rat synaptosomal membranes were pretreated with pentobarbital and then the basal, Mn^{2+}- and forskolin-stimulated adenylate cyclase activities were measured. Figure 1 shows that pentobarbital inhibits the enzyme activities in a dose-dependent manner. At 2.5 mM pentobarbital, the inhibition of the basal and Mn^{2+}-stimulated enzyme activities is approximately 25%, whereas the inhibition of forskolin-stimulated enzyme activity is approximately 40%, suggesting that this enzyme system activated by forskolin is more sensitive to pentobarbital. Although millimolar concentrations of pentobarbital are required to inhibit the enzyme activity, the concentrations are coincident with those previously reported by Lohse *et al.* who observed that only higher concentrations of pentobarbital inhibited both basal and 5′-*N*-ethylcarboxamidoadenosine-stimulated adenylate cyclase activities of NIE 115 neuroblastoma cells.[16]

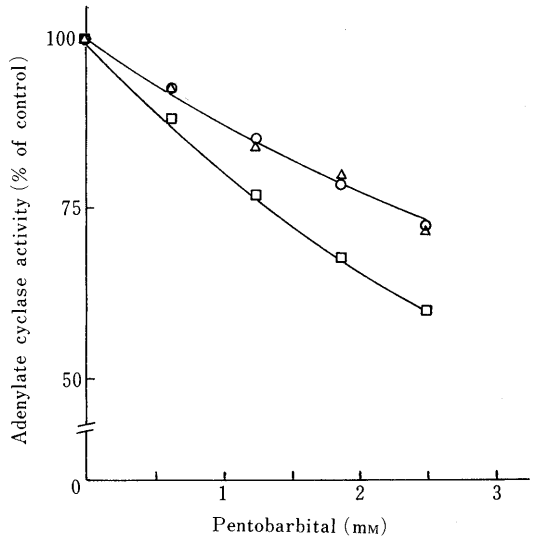

Fig. 1. Inhibition of Adenylate Cyclase Activity in Synaptosomal Membranes by Pentobarbital

The synaptosomal membranes were pretreated with the indicated concentrations of pentobarbital in 125 mM Tris–HCl buffer (pH 7.4) for 2 h at 30 °C, and then assayed for the basal (○), 10 μM forskolin (□)- and 2.5 mM $MnCl_2$ (△)-stimulated adenylate cyclase activities. The basal, forskolin-, and $MnCl_2$-stimulated adenylate cyclase activities without pentobarbital were 37.2, 197.1 and 150.7 pmol/min/mg protein, respectively.

TABLE I. Comparison of the Inhibitory Effects of Pentobarbital on Adenylate Cyclase Activity in Brain and Peripheral Tissues

Tissues	Adenylate cyclase activity (pmol/min/mg protein) Control		Pentobarbital		Inhibition[a] (%)	
	Basal	Forskolin	Basal	Forskolin	Basal	Forskolin
Brain	30.5±0.9	151.7±1.9	21.4±0.3	85.9±1.8	29.8	43.4
Heart	7.1±0.2	54.6±2.3	3.6±0.2	26.9±1.1	49.3	50.7
Kidney	1.3±0.1	8.3±0.4	0.8±0.1	4.9±0.2	39.7	40.5

Rat heart and kidney plasma membranes and synaptosomal membranes were pretreated with or without 2.5 mM pentobarbital for 2 h at 30 °C, and then adenylate cyclase activity was assayed in the presence or absence of 10 μM forskolin. Values are the mean±S.D. ($n=3$).

a) Inhibition percent of the activity was calculated as follows:

$$\frac{(\text{activity without pentobarbital}) - (\text{activity with pentobarbital})}{(\text{activity without pentobarbital})} \times 100$$

Furthermore, we examined if pentobarbital inhibited forskolin-stimulated adenylate cyclase activity more effectively than that in the basal state in peripheral tissues. The plasma membranes from rat heart and kidney were pretreated with 2.5 mM pentobarbital and then the basal and forskolin-stimulated enzyme activities were measured. Table I showed that pentobarbital inhibited both basal and forskolin-stimulated enzyme activities in peripheral tissues. As far as the basal activity is concerned, our findings seem to be in line with the reported results that barbiturates inhibited adenylate cyclase activity from guinea pig heart and lung.[17] But, no significant difference was observed in the degree of the inhibition by pentobarbital between the basal and forskolin-stimulated activities. The inhibitions of both activities in the plasma membranes from heart and kidney were approximately 50% and 40%, respectively. The degree of inhibition is consistent with that in the presence of forskolin in synaptosomal membranes. These findings clearly indicate that the inhibitory effect of pentobarbital on the basal adenylate cyclase activity is specifically low in the central nervous system and that forskolin auguments the magnitude of the inhibitory effect of pentobarbital on the enzyme activity. In other words, it is suggested that the conformation of the catalytic unit of rat brain adenylate cyclase system is different from that in the peripheral tissues, whereas the presence of forskolin induces conformational changes of the catalytic unit to increase the sensitivity to inhibition by pentobarbital.

To further elucidate that the inhibition of forskolin-stimulated adenylate cyclase activity by pentobarbital, we studied the forskolin concentration dependency using synaptosomal membranes pretreated with 2.5 mM pentobarbital. Table II shows that the inhibition by pentobarbital of the enzyme activity is augumented by the presence of forskolin in a concentration-dependent manner. In addition, the inhibition by pentobarbital is saturable at approximately 1.0 μM forskolin, although we have no explanation as to why 40% inhibition is maximal.

Recent reports demonstrate that forskolin binds to two sites in the adenylate cyclase system, a low affinity binding site, which is responsible for the direct activation of the catalytic unit and a high affinity binding site, which is involved in the coupling with the catalytic unit and activated G_s in rat brain[18] and human platelet.[19] Additionally, we recently indicated that forskolin stabilized a functionally coupled state between activated G_s and the catalytic unit of the adenylate cyclase system in rat erythrocytes,[5] suggesting that pentobarbital primarily affected a high affinity binding site for forskolin. The use of pentobarbital may be useful for further investigation of the action of forskolin on the adenylate cyclase system in the central nervous system, although it remains to be elucidated whether the inhibitory effect of pentobarbital on the adenylate cyclase system and the augumentation of the sensitivity to pentobarbital by the presence of forskolin in the central nervous system are actually related to the anticonvulsant effect of pentobarbital.

TABLE II. Inhibitory Effect of Pentobarbital on Adenylate Cyclase Activity in the Presence of Various Forskolin Concentrations in Rat Synaptosomal Membranes

Forskolin (μM)	Adenylate cyclase activity (pmol/min/mg protein) Control	Pentobarbital	Inhibition[a] (%)
0	30.5±0.9	21.4±0.3	29.8
0.1	50.0±1.3	31.9±0.6	36.2
0.5	80.0±0.7	48.7±1.4	39.5
1.0	98.0±1.1	57.7±1.9	41.1
5.0	136.0±2.4	79.0±1.8	41.9
10.0	151.7±1.9	85.9±1.8	43.4

The synaptosomal membranes were pretreated with or without 2.5 mM pentobarbital for 2 h at 30 °C, and then adenylate cyclase activity was assayed in the presence of the indicated concentrations of forskolin for 20 min at 30 °C. Values are the mean±S.D. ($n=3$). *a*) The inhibition of the activity was calculated in the same way as in Table I.

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