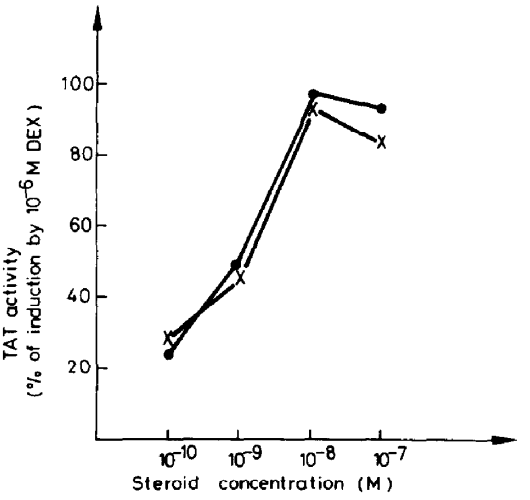

Fig. 1. Induction of TAT by dichlorison (X——X) and 16α-methyl dichlorison (○——○) in Fao cells. Monolayer cultures were exposed to varying concentrations of steroids for 18 hr and harvested for determination of TAT specific activity as previously described [7, 8]. The increase in TAT activity above the basal level was plotted as per cent of the TAT activity induced by 10^{-6} M dexamethasone. Sp. act.: 20.65 (basal), 189.75 (induced by 10^{-6} M DEX). Concentration of DEX required for half-maximum induction of TAT is: 1 nM. Each point represent the average of 2–4 determinations.

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Reduced cyclic-AMP responsiveness in the colliculus inferior of audiogenic seizure-prone rats

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A relationship between cyclic AMP (cAMP) and epileptic seizure activity has been demonstrated in a large number of experiments. Accumulation of cAMP has been observed in the brain electric-shock treatment [1, 2] or following administration of several convulsant drugs [3–7]. The concentration of cAMP was also increased in epileptic foci [8]. In addition, it has been found that dibutyryl cAMP injected intracerebrally alters seizure threshold, however, data on this point are contradictory [9–11].

Although it is generally accepted that genetic factors are involved in the development of human epilepsy, the possible role of cAMP in epileptic mechanisms has not been studied in genetically epilepsy-prone animals. Although many neurochemical parameters involved in seizure-prone animals have been extensively investigated [12–14], no data is available on the function of cAMP in this regard. Therefore the aim of this paper was to clarify whether there is any difference between epilepsy-prone and normal animals in their basal and stimulated brain cAMP content. Since stimulation of the cAMP system of the brain during pentylenetetrazol (PTZ)-induced seizures is well described [3, 4], PTZ was used to test the capacity of the cAMP apparatus for increased synthesis.

According to large number of publications audiogenic seizure-prone rats (ASPR) are good objects for examining a genetic disposition to epilepsy [12–14]. The relative neutrality of the cortex temporalis (CT) and the important role of the colliculus inferior (CI) in the development of audiogenic convulsions have also been shown [15, 16]. Therefore basal and stimulated cAMP levels in CT and CI of ASPR and normal rats were determined in these experiments.

Methods

Male CFY rats (180–240 g) were used in the experiments. Separation of ASPR from controls was made in a special

cylindrical sound chamber, which has been described previously [14]. Rats were considered ASPR if in response to a standardized-sound stimulus they exhibited a generalized clonic-tonic convulsion in two separate tests conducted at 4 day intervals. Measurement of cAMP was made 4 days after selection. Animals were sacrificed by means of focused microwave irradiation of the brain (Gerling Moore Inc., 2450 MHz, 2 sec). After the removal of the brain, CT and CI of both hemispheres were dissected and frozen immediately on dry ice. For determination of cAMP concentrations samples were extracted by the method of Schmid *et al.* [17]. Cyclic AMP was measured radioimmunologically according to Brooker *et al.* [18]. Concentrations were expressed in pmoles/mg wet wt of tissue. Pentylenetetrazol (Chinoin, Budapest) was given i.p. (100 mg/kg) and rats were killed 4 min after injection. This dosage of PTZ and exposure time have been shown to sufficient for stimulation of cAMP [3, 4]. Since difference was observed in cAMP response between ASPR and normal rats (see Results), cAMP concentration 1 and 2 min following PTZ injection was also measured in an other experiment. Student's *t*-test and two way of analysis of variance were used for statistical evaluation.

Results

In basal cAMP levels measured in either CT or CI there was no significant difference between ASPR and control rats. The cAMP concentration in the CT was 0.64 ± 0.07 pmoles/mg ($N = 18$) in ASPR and 0.75 ± 0.08 pmoles/mg ($N = 18$) in the controls ($P > 0.05$). The corresponding values in the CI were 0.76 ± 0.07 pmoles/mg ($N = 18$) in the ASPR and 0.58 ± 0.06 pmoles/mg ($N = 18$) the control group, which are also statistically insignificant ($P > 0.05$).

Following PTZ administration, clonic-tonic convulsions developed in each group of animals. Within 4 min after injection all of the animals were in the tonic phase. Dyspnoe, a characteristic hypoxic symptom was not seen at this time. A concentration of 100 mg/kg of PTZ caused death 9–10 min after injection, as was shown in a separate test. Pentylenetetrazol induced seizures resulted in a significant increase of cAMP levels of CT in both groups. Four minutes after injection of the drug, the concentration of the cAMP was 1.15 ± 0.14 pmoles/mg ($N = 14$) in the control rats and 0.94 ± 0.12 pmoles/mg ($N = 14$) in the ASPR. These increases (153 and 147% of the basal values) were significant ($P < 0.05$). Surprisingly, in the CI the accumulation of cAMP following PTZ administration was detectable only in the control rats. In these animals cAMP was raised to 0.93 ± 0.10 pmoles/mg ($N = 10$). This elevation (158% of basal value) is significant ($P < 0.05$). In contrast, in ASPR PTZ treatment did not change the cAMP level of CI, it was 0.79 ± 0.12 pmoles/mg ($N = 10$). These results are summarized in Fig. 1. Studying the time-dependency of the altered cAMP response in the colliculus inferior, similar data were obtained. While in normal rats the cAMP concentrations measured 1 and 2 min after PTZ injection were 172 and 176% of the basal value, in ASPR there was not any increase in the cAMP content, even a slight but not significant decrease (75 and 82% of the basal value) was observed.

Discussion

Our experiments indicate that although there is no significant difference in basal cAMP concentration of CT and CI of ASPR and normal rats, changes in cAMP levels in response to PTZ administration are rather different. Data presented in this paper show a reduced responsiveness of cAMP to PTZ exposition in the colliculus inferior of genetically audiogenic seizure-prone rats. Since this area plays an important role in proneness to audiogenic seizure, this deficiency might be characteristic of the disturbance. The fact that either basal or stimulated cAMP level values were similar in the cortex temporalis of the two groups of rats might be explained by the relative neutrality of this area in the pathomechanism of audiogenic convulsions.

In summary, a reduced responsiveness of the cAMP system could be detected in the colliculus inferior of genetically audiogenic seizure-prone rats. This phenomenon might be in connection with the genetic anomaly of ASPR, and support the hypothesis [4, 11] that cAMP may exert a protective effect against the propagation of seizures, since its deficit could be measured in seizure-prone animals.

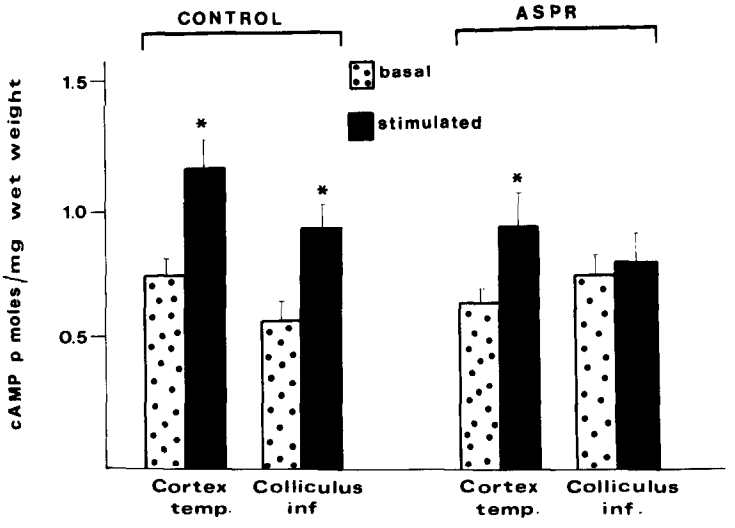

Fig. 1. Basal and stimulated cAMP levels in the cortex temporalis and colliculus inferior of normal rats and ASPR. Pentylenetetrazol (100 mg/mg i.p.) was used for stimulation of cAMP. Exposure time of the drug was 4 min. Each bar represents mean ± S.E.M. (14–18 animals were used in every case). *Indicates significant difference from basal value ($P < 0.05$).

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Ginseng saponin treatment does not alter brain or pituitary levels of beta-endorphin and dynorphin

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The root of *Panax ginseng* has been used extensively in oriental communities as a vitalizing agent to counteract the deterioration of bodily functions accompanying old age. Scientific investigations on the pharmacology and biochemistry of ginseng have begun only in recent years. More than twelve ginseng saponins have been isolated [1–4], and these compounds have been shown to have a variety of metabolic actions ranging from stimulation of protein and RNA synthesis in the liver [5] to increase of cAMP level in the adrenal gland [6] and stimulation of ACTH release from the pituitary [7]. In addition to these biochemical changes, administration of ginseng to experimental animals can induce a number of physiological effects such as prevention of body temperature fluctuation after hot and cold exposure [8], improved exercise rates and prolonged swimming times [9], and reduction in radiation damage [10].

Although the experimental evidence gathered so far has provided some support for the pharmacological action of ginseng, the scientific establishment of how it can mediate all the actions claimed, no doubt, requires more detailed and careful studies. The opioid peptides have been shown to be involved in a number of neurological functions; the most noteworthy of which are pain and behaviors. Since an increase in stress tolerance is frequently claimed after intake of ginseng [9], an augmented release of the opioid peptides may be the underlying cause. In the experiment reported in this study, we examined the effect of ginseng administration on the levels of β-endorphin and dynorphin A in the hypothalamus and the pituitary gland.

Materials and methods

A ginsenoside (ginseng saponin) fraction, prepared according to the method of Sanada *et al.* [3] as modified by Yeung *et al.* [11] was used in this study. Briefly, pulverized lateral roots were extracted three times with hot methanol (2 ml/g) and after evaporation of the solvent under reduced pressure, the residue was suspended in water and washed with ether. The aqueous fraction was then extracted with *n*-butanol saturated with water. The *n*-butanol layer was concentrated *in vacuo* to give the ginsenoside fraction with a yield of 11%. The potency of this preparation was independently confirmed by its suppressive action on delayed-type of hypersensitivity [11]. Male Sprague–Dawley rats weighing between 160 and 200 g were used in this study. Animals were housed in a temperature-controlled room at 25° with 12 hr of artificial light starting from 0600 hr. Food and water were freely accessible. Treatment with ginseng was initiated after a stabilization period of three days. Rats were injected intraperitoneally in the morning with either a 1 ml saline solution or ginseng extract dissolved in the same volume of saline. The dose used was 3 mg/kg body weight per day for three consecutive days. No adverse reaction was noticed after ginseng administration. Rats were sacrificed by decapitation 24 hr after the last injection. Their brains and pituitary glands were quickly removed, chilled and dissected on ice. Extraction of opioid peptides was carried out immediately afterwards according to the procedure of Rossier *et al.* [12]. Briefly, the tissue was first incubated in 1 N acetic acid (10 ml for hypo-