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3'-5' CYCLIC-GUANOSINE MONOPHOSPHATE INCREASE IN RAT BRAIN HIPPOCAMPUS AFTER GAMMA-HYDROXYBUTYRATE ADMINISTRATION. PREVENTION BY VALPROATE AND NALOXONE.

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Summary

An increase (123%) of cyclic GMP (cGMP) was observed in the hippocampus of the rat killed by microwave irradiation 45 min after administration of 500 mg/kg γ-hydroxybutyrate (GHB) IP. This increase is time and dose dependent. No modification in cyclic nucleotide content was observed in striatum and in cerebellum. As the role of GHB has been implicated in neurotransmission, the fact that this compound increases cyclic GMP accumulation in hippocampus in vivo may represent a mechanism by which the actions of GHB are mediated at the cellular level. Valproate (400 mg/kg) or naloxone (10 mg/kg) pretreatment completely abolish the cGMP increase due to GHB. A GABAergic and/or opiate phenomenon may be involved in the mechanism of GHB induced increase of cGMP.

An increase of adenosine 3'-5' cyclic-adenosine-monophosphate (cAMP) or of 3'-5' cyclic-guanosine-monophosphate (cGMP) or both have been observed after administration of several convulsant drugs and agents in experimental animals (1,2). Moreover, agents that lead to behavioral excitation tend to increase cGMP levels whereas those that depress motor activity decrease its levels (2). Interestingly, γ-hydroxybutyrate (GHB) which occurs naturally in the brains of several mammalian species (3) including man (4), induces when administered to animals a state of behavioral sedation often called sleep or anaesthesia (5). In addition, GHB induces hypersynchronism in the electroencephalographic pattern in rat, rabbit and man (6,7,8). These effects have been described as epileptoid E.E.G. seizures which can be antagonized by anti-petit mal drugs. Besides these effects, GHB is a good candidate for a role in neurotransmission or neuromodulation (9). The cyclic nucleotides, involved in the cellular action of numerous neurotransmitters, can also mediate the neuroregulatory effects of GHB in mammalian brain. The aim of this paper is to investigate the effect of exogenous GHB on the level of cyclic nucleotides in three regions of the rat brain : hippocampus, which is considered as the burst generator for several acute epilepsy models (10,11), cerebellum, where cyclic nucleotides have been extensively studied (12,13,14), and striatum where GHB interacts with the dopaminergic system (8-15).

Materials and Methods

Male adult Wistar rats weighing about 300 g were used in all studies. The animals were injected IP with GHB (sodium salt) and/or with the other test substances (sodium valproate, or naloxone hydrochloride). The rats were sacrified after appropriate times by exposing the head to focused microwave irradiation (7.5 kW, 1.6 sec. exposure) which prevents post-mortem changes in cyclic nucleotide levels (16). The dissected brain regions were kept in liquid

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nitrogen, weighed frozen and homogenized in 10 vols 1M ice cold perchloric acid. Protein was removed by centrifugation at 20,000 g for 25 min. The supernatants were neutralized with 3M K_2CO_3 and cyclic nucleotide contents were determined with the cAMP kit and the cGMP R.I.A. kit from Amersham (Radiochemical Center). Protein contents of the different pellets were measured by the Lowry method (17) after solubilization in 2N NaOH.

Results

Cyclic nucleotides as a function of time after GHB administration

Cyclic nucleotide levels were measured every 10 min during 120 min after injection at time zero of 500 mg/kg GHB. No significant changes were found for cGMP and cAMP in the cerebellum or in the striatum. (cGMP: cerebellum (3.02 ± 0.48 pmole/mg protein), striatum (0.25 ± 0.06 pmole/mg protein); cAMP: cerebellum (6.29 ± 0.53 pmole/mg protein), striatum (3.14 ± 0.56 pmole/mg protein). In the hippocampus, the level of cGMP (0.28 ± 0.05 pmole/mg protein) increases with time. The rise of cGMP was first noted 20 min after injection of GHB, with a maximum at 30-50 min (0.63 ± 0.04 pmole/mg protein). After 110 min, the basal level of cGMP is restored (Fig. 1). For cAMP, no significant changes were found (3.57 ± 0.96 pmole/mg protein).

Effect of various concentrations of GHB on cGMP levels in hippocampus

200 mg/kg to 700 mg/kg GHB were administered IP to rats which were killed after 45 min by microwave irradiation. cGMP levels were determined in the dissected hippocampus. Fig. 2 shows that the maximum increase in cGMP occurs for 400 - 500 mg/kg. Higher doses induced less accumulation of cGMP.

Effect of valproate on the cGMP increase induced by GHB in hippocampus

The animals were injected either with valproate (400 mg/kg IP) or with GHB (400 mg/kg IP) or pretreated with valproate (400 mg/kg IP) 15 min before GHB injection (400 mg/kg IP). In all cases, 45 min after the last injection, the animals were killed as described above and cGMP was determined in the hippocampus. Fig. 3 shows that valproate does not modify cGMP content, but GHB increases cGMP in hippocampus by about 140% compared to controls injected with saline. However, pretreatment with valproate completly abolishes the GHB effect on cGMP levels (Fig. 3). Under these conditions, no modifications of cGMP levels are observed compared to controls injected with saline or with valproate alone.

Effect of naloxone on the cGMP increase induced by GHB

For these experiments, the same protocol as described for the experiment with valproate was adopted, but this latter compound was replaced by administration of naloxone (10 mg/kg IP). As indicated by SNEAD et al. (19) naloxone completely blocked behavioral changes induced by administration of GHB. In particular, no catalepsy was observed in animals receiving both naloxone and GHB. Pretreatment with naloxone blocks the GHB effect on cGMP levels (Fig. 4).

Discussion

This work demonstrates the increase of cGMP accumulation induced by GHB in rat brain hippocampus. The control values of cGMP are identical to those previously described for hippocampus of rats sacrificed by microwave irradiation (18). No changes were found in the other regions studied either for cGMP or for cAMP levels. GHB caused a time and dose dependent accumulation of

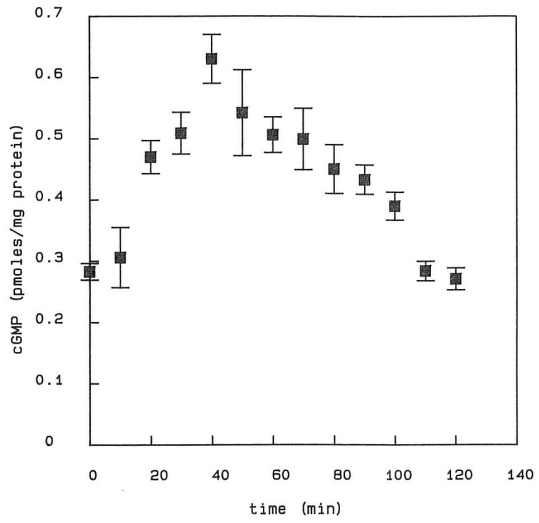

FIG. 1

cGMP levels in hippocampus as a function of time after GHB administration (500 mg/kg IP). Each point represents the mean of 3 different determinations ± S.E.M.. The cGMP levels between 20 and 100 min are significantly different from the control with $p < 0.05$ (Student's t test).

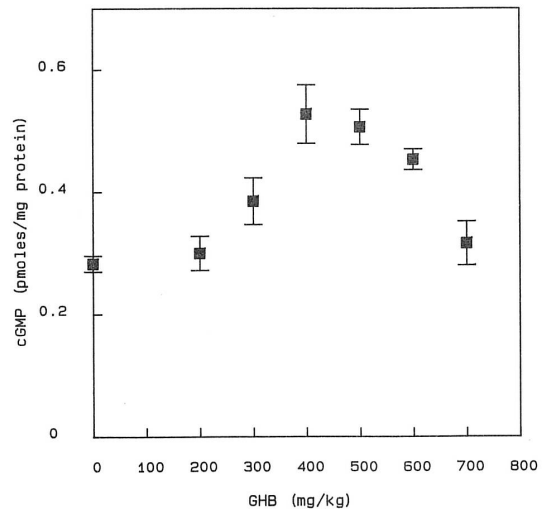

FIG. 2

Effect of various GHB doses on cGMP levels in hippocampus. The rats were killed 45 min after GHB injection IP. Each point represents the mean of 3 different determinations ± S.E.M. The cGMP levels between 400 and 600 mg/kg are significantly different from the control with $p < 0.05$ (Student's t test).

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