

EFFECT OF ETHIMIZOLE ON CONTENT OF CYCLIC AMP IN BRAIN TISSUE

I. S. Zavodskaya, É. A. Migas,
and V. V. Bul'on

UDC 615.214.31.015.45:612.82.015.2:547.963.32

It was shown by the use of an enzymic method of determining cyclic 3',5'-adenosine monophosphate (cyclic AMP) that ethimizole more than doubles the concentration of this substance in the rat brain tissue 20 min after intraperitoneal injection in a dose of 25 mg/kg. Characteristic changes of carbohydrate metabolism associated with an increased cyclic AMP were found in the brain: a decrease in the glycogen content and an increase in the glucose content. It is concluded that the mechanism of action of ethimizole is connected with increased formation of cyclic AMP in brain tissue.

Key words: brain; ethimizole; carbohydrate metabolism.

The great therapeutic value of ethimizole is connected with its action on brain structures with many different functions. However, the biochemical mechanism of the activity of ethimizole has received little study.

Investigations in the writers' laboratory, under Academician of the Academy of Medical Sciences of the USSR S. V. Anichkov's direction, have shown that ethimizole has a marked activating action on brain adenylyl cyclase. On this basis it might be expected that ethimizole would cause an increase in the content of cyclic 3',5'-adenosine monophosphate (cyclic AMP) in the brain tissue and associated metabolic changes, for cyclic AMP is known to be a cofactor for many enzyme systems.

The object of this investigation was to study the effect of ethimizole on the cyclic AMP level in rat brain tissue and also on the levels of glycogen and glucose, the principal substrates of carbohydrate metabolism, whose content is also dependent on cyclic AMP.

EXPERIMENTAL METHOD

Ethimizole was injected intraperitoneally into male rats in a dose of 25 mg/kg. Brain tissue was taken for investigation 20 min later.

Cyclic AMP was determined by an enzymic method [2, 5]. The brain tissue was frozen in liquid nitrogen and homogenized in 5 volumes of 10% TCA. Cyclic AMP was separated from the other adenosine nucleotides by adsorption on $\text{ZnSO}_4\text{-Ba(OH)}_2$, followed by high-voltage electrophoresis on paper [8]. The volume of supernatant taken for electrophoretic fractionation was 0.1 ml. Cyclic AMP was identified by its ultraviolet fluorescence and eluted with 50% ethanol, which was then removed by means of a vacuum evaporator.

Each sample was then treated with 0.2 ml 0.5 M Tris-HCl buffer, pH 7.7, and 1 mM MgSO_4 . The cyclic AMP was then converted into 5'-AMP by the addition of 0.01 ml (20 μg) 3',5'-AMP phosphodiesterase (purified lyophilized preparation from bovine heart with an activity of 0.45 unit/mg; Sigma, USA) to 0.05 ml of the solution of cyclic AMP. The reaction was stopped by boiling after incubation for 30 min at 37°C.

Laboratory of Experimental Pharmacology, Department of Pharmacology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR S. V. Anichkov.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 79, No. 3, pp. 54-56, March, 1975. Original article submitted April 17, 1974.

© 1975 Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for \$15.00.

TABLE 1. Content of Cyclic AMP, Glycogen, and Glucose in Brain Tissue of Rats 20 min after Administration of 25 mg/kg Ethimizole ($M \pm m$)

Parameter	Control	Ethimizole given
Cyclic AMP (in moles/g)	$1,1 \pm 0,2 \cdot 10^{-9}$ (5)	$2,4 \pm 0,2 \cdot 10^{-9}$ (5)
Glycogen (in mg %)	$68,0 \pm 2,6$ (10)	$29,0 \pm 1,6$ (10)
Glucose (in mg %)	$28,6 \pm 0,8$ (7)	$37,5 \pm 2,2$ (7)

Legend. Number of determinations shown in parentheses.

The samples were cooled to 0°C and this was followed by the addition of an ATP-generating system, consisting of 0.15 ml of reagent containing 0.2 M Tris-HCl buffer, pH 7.5, 6 mM MgCl₂, 0.1 M KCl, 15 mM di-nitrothreitol, 7.5 mM phosphoenolpyruvate, 0.5 mM EDTA, 1×10^{-8} M ATP, 30 µg/ml myokinase (preparation from pig heart with activity of 940 units/mg; Sigma), and 80 µg/ml pyruvate kinase (from rabbit muscle, activity 100 enzyme units/mg; Reanal, Hungary). The samples were allowed to stand overnight at room temperature, after which an ATP-utilizing system was added: 0.1 ml of a reagent containing 10 mg/ml hexokinase (Cyclo, USA) and 60 mM glucose. After incubation for 1 h at 37°C the reaction was stopped by boiling. The samples were cooled and centrifuged, after which 0.01 ml of the supernatant was added to 4 ml of a solution containing 0.1 M Tris-HCl buffer, pH 7.5, 0.1 mM NADP, and 1 µg/ml glucose-6-phosphate dehydrogenase (Fluka, Switzerland). The NADP·H₂, formed in an amount corresponding to the ATP and, consequently, the cyclic AMP content in the samples, was measured fluorometrically with the BIAN-130 fluorometer.

To determine the glucose and glycogen in the tissue the rat brain was frozen in liquid oxygen. Precipitation of the glycogen and its purification were carried out by the method of Kerr [6]. The glucose content after hydrolysis of the glycogen residue was determined enzymically by the glucose oxidase method [1]. The glucose content in the brain tissue was determined by the hexokinase method [9].

EXPERIMENTAL RESULTS

Determination of cyclic AMP by the enzymic method showed that its concentration in rat brain tissue was 1.1×10^{-9} mole/g tissue, in agreement with data in the literature: according to Breckenridge [2], the cyclic AMP content of mouse brain tissue is 0.8×10^{-9} – 1.2×10^{-9} mole/g tissue, and according to Goldberg et al. [5], the cyclic AMP content in rat brain tissue is 2.2×10^{-9} mole/g tissue.

The results of the present experiments showed that 20 min after its administration in a dose of 25 mg/kg, ethimizole more than doubled the cyclic AMP content in the brain tissue compared with the control (Table 1). The brain glycogen level under the same conditions fell considerably (29.0 mg%, normal 68.0 mg %). The average increase in the glucose content was 30%, a significant change compared with the control.

Ethimizole thus leads to marked accumulation of cyclic AMP in brain tissue accompanied by an increase in the glucose and a decrease in the glycogen content.

The changes thus revealed in the glycogen and glucose content in the brain under the influence of ethimizole are characteristic of an increase in the cyclic AMP content. One of the most important effects of cyclic AMP on carbohydrate metabolism is its glycolytic action. Cyclic AMP activates the kinase "B" of phosphorylase which, in turn, converts the phosphorylase from the inactive into the active state [7]. Many investigations have shown an increase in glucose production in the tissues following an increase in the cyclic AMP concentration, and this may come about in three ways: by activation of phosphorylase, by inactivation of glycogen synthetase, and by stimulation of gluconeogenesis [3, 4, 10].

An increase in the cyclic AMP content in the brain, confirmed by the direct determination of cyclic AMP and indirectly, by characteristic changes in carbohydrate metabolism, was thus demonstrated under the influence of ethimizole in the brain. The writers previously showed that ethimizole stimulates adenyl cyclase activity. It can accordingly be concluded that the molecular mechanism of the activating action of ethimizole is connected with increased formation of cyclic AMP in the brain.

LITERATURE CITED

1. I. S. Lukomskaya and V. K. Gorodetskii, *Biokhimiya*, 26, 477 (1961).
2. B. Breckenridge, *Proc. Nat. Acad. Sci. (Washington)*, 52, 1580 (1964).
3. J. Exton and C. Park, *Pharmacol. Rev.*, 18, 181 (1966).
4. D. Friedman and J. Larner, *Biochim. Biophys. Acta*, 64, 185 (1962).
5. M. Goldberg, J. Larner, H. Sasko, et al., *Analyt. Biochem.*, 28, 523 (1969).
6. S. Kerr, *J. Biol. Chem.*, 116, 1 (1936).
7. J. Posner, R. Stern, and E. Krebs, *Biochem. Biophys. Res. Commun.*, 9, 293 (1962).
8. R. Sandhu and M. Hokin, *J. Chromatogr.*, 35, 136 (1968).
9. M. Slein, in: *Methoden der enzymatischen Analyse*, Weinheim (1962), p. 117.
10. T. Soderling and J. Hickenbottom, *Fed. Proc.*, 29, 601 (1970).