Regional cyclic AMP levels in homogenates of rat brain after ketalar and trifluoperazine[1]

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Summary. There was a significant fall in cAMP levels after administration of TFP or ketalar. Different amounts of cAMP were present in different regions of rat brain. Concentrations of cAMP in different regions of the rat brain were found to decrease in the following order: cerebrum > thalamus with hypothalamus > midbrain > hippocampus > cerebral cortex.

Brain tissues have particularly high activities of adenylate cyclase (AC-ATP pyrophosphate-lyase/cyclizing/EC 4.6.1.1) and cyclic nucleotide phosphodiesterase (PDE-3′: 5′-cyclic nucleotide phosphodiesterase EC 3.1.4.1), and possess relatively high concentrations of cAMP (adenosine cyclic 3′: 5′-monophosphate). It has been shown that several neuronal and hormonal factors influence the activity of AC[2-4].

The studies with catecholamine-induced activation of AC suggested a link between cAMP content and specific types of synaptic transmission[5-10]. The concentration of cAMP increases in brain following decapitation. Endogenously released catecholamines and the anoxia accompanying decapitation have both been suggested as probable causative agents[3].

Using this postdecapitation rise of cAMP as a model for activating the cAMP system in several regions of rat brain we have studied whether the administration of some anaesthetic drug such as ketamine hydrochloride (ketalar), and similarly trifluoperazine (TFP), could alter the rise of cAMP in certain regions of brain following decapitation.

Materials and methods. TFP, as triftazine Thera Plix, administered i.p. to rats 60 min before decapitation at doses of 30 μmoles/kg. Ketalar (Parke-Davis) was administered at doses of 10 mg/kg i.m. Male rats of F_1 generation, weighing 300–400 g and bred from Buffalo and Wistar females were used. The animals were killed by cervical fracture and decapitation, and the brains without cerebellum were removed immediately into ice-cold medium (10 mmoles NaCl + 10 mmoles KCl).

The brains were dissected into 4 regions: 1. cerebral cortex; 2. hippocampus; 3. midbrain; 4. thalamus with hypothalamus, and stored in dry ice. The homogenization was carried out using 10 strokes with a hand-operated Potter-Elvehjem homogenizer.

cAMP was assayed by saturation analysis using binding protein[11], according to the procedure of Gilman[12], applying a cyclic (^{3}H) AMP assay kit (Amersham). The extraction was carried out with HCl in a water bath at 100 °C for 2 min[13]. This method has been chosen as the best one for the elimination of interfering factors present in the brain extracts. Protein was measured as described by Lowry et al.[14], using bovine albumin as a standard.

Results and discussion. In rats the content of cAMP in crude homogenates obtained from different areas of the brain differs substantially. Data obtained by us (table) are in agreement with those published by Palmer[2].

The administration of an anaesthetic (ketamine HCl) or psychotolitic (TFP) prior to the decapitation produces a reduction in concentration of cAMP in comparison with values obtained on animals decapitated without such treatment. Administration of ketalar resulted in our experiments in a decrease in the concentration of cAMP in homogenates of all 4 areas of the brain. It was maximal in the thalamus with hypothalamus (53%). After TFP the maximal decrease of concentration (85%) was observed in the midbrain. The smallest decrease was noticed in cerebral cortex following the administration of both drugs, ketamine or TFP.

The decrease of the concentration of cAMP after administration of TFP was 58–85% in all 4 regions of the brain investigated. Ketamine was less active in producing this effect as compared with TFP; it was 20–53%. The mechanism of the action of anaesthetics on the metabolism of the brain is still unknown, owing to the insufficient experimental data.

Ketamine is chemically quite different from other anaesthetics and has different pharmacological action[15]. The activity of other anaesthetics therefore cannot serve as a guide for the understanding of the activity of ketamine. Various effects of ketamine on brain tissue[16-18] include the dual influence on the CNS: 1. depression of the thalamoneocortical system, and 2. stimulation of hippocampal firing[19-21].

Assuming that ketamine exerts in the brain only an incomplete depressive action it might be supposed that there occurs a partial decrease of the rate of synthesis of some stimulators of cerebral activity (e.g., some biogenic amines) and/or inhibition of their release. These effects might be caused by a depressed neuronal activity in the brain.

On the other hand, the formation of cAMP in the brain tissue appears to be regulated by several parameters: e.g., by the concentration of biogenic amines such as nor-

Effect of ketalar (K) and trifluoperazine (TFP) on the concentrations of cAMP (mean ± SD) in several areas of rat brain

	Decapitation (n = 10)	Ketalar, 10 mg/kg 60 min before decapitation (n = 7)	TFP, 30 μmoles/kg 60 min before decapitation (n = 5)	Significance (Student's t-test)
Cerebrum	30.60 ± 4.26	5.61 ± 0.90	4.54 ± 1.03	K/D $p > 0.001$ TFP/D $p > 0.001$
Cerebral cortex	13.82 ± 2.05	8.49 ± 1.30	3.46 ± 0.85	$p > 0.02$ $p > 0.01$
Hippocampus	16.31 ± 2.76	9.05 ± 1.40	4.13 ± 0.90	$p > 0.05$ $p > 0.01$
Midbrain	21.68 ± 3.26	9.23 ± 2.00	2.06 ± 0.60	$p > 0.01$ $p > 0.001$
Thalamus with hypothalamus	23.87 ± 3.55	8.08 ± 2.09	5.46 ± 1.39	$p > 0.01$ $p > 0.01$

Cyclic AMP levels are expressed as pmoles/mg protein.

epinephrine, histamine, 5-hydroxytryptamine (serotonin) and others. It was shown that ketamine produced a decrease of the concentration of serotonin in the brain of rats[22]. This possible decrease of serotonin could result the decrease in the stimulation of cAMP formation reported in our experiments. It is necessary to notice that there is conformity between the maximal depressive effect of ketamine on the thalamus system, described in the literature, and the maximal decrease of cAMP concentration in the same region reported in our study.

Further studies will be required to elucidate these possibilities, especially in relation to the kinetics of accumulation and elimination of ketamine in various areas of the rat brain.

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Glutamine synthetase activity in subdivisions of brain of the shark, *Squalus acanthias*[1]

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Summary. Specific activity of glutamine synthetase in *Squalus acanthias* (spiny dogfish) central nervous system regions was highest in the cerebellum and lowest in the spinal cord. The levels of activity may relate to the excitability of each region by regulating the glutamate pool.

The only known physiologically significant route of glutamine synthesis in all species is the activity of L-glutamate:ammonia ligase (ADP-forming) (EC 6.3.1.2)[2]. Presence of this enzyme in brain tissue has been known for some time[3,4]. Waelsch[5] suggested that glutamine synthetase regulates the glutamate pool in the brain. The proposed function of glutamate as an excitatory transmitter has been reviewed previously[6,7]. An ammonia detoxification role has also been considered for mammals[8] and for fish[9,10]. The recent finding that the vast majority of glutamine synthetase activity in rat brain is restricted to glial cells[8] may help to clarify the role of glutamine synthetase in the brain. As part of a comparative study of glutamine synthetase in fish tissues[11,12], regions of *Squalus acanthias* (spiny dogfish) brain were examined to determine whether activity was localized in a specific area of the central nervous system.

Brain tissue was excised from freshly sacrificed, female *Squalus acanthias* (length: 87–105 cm). Subdivision and identification of brain regions was according to Gilbert[13] and Smith[14]. The γ-glutamyl hydroxamate standard, L-glutamine, NaADP, imidazole, KH_2AsO_4, and bovine serum albumin were obtained from Sigma Chemical Co., St. Louis, Mo. Hydroxylamine-HCl was obtained from Merck and Co., Rahway, N.J.

The freshly excised brain segments were homogenized with 9 volumes of water in glass, hand homogenizers at 0–5 °C and assayed within 30 min. The assay for glutamine synthetase activity followed the depicted reaction as described by Webb and Brown[11].

$$\text{L-glutamine} + NH_2OH \xrightarrow[AsO_4^{3-}]{\text{ADP } Mn^{++}} \gamma\text{-glutamyl hydroxamate} + NH_3$$

Glutamine synthetase activities and protein content: regions of *Squalus acanthias* brain and spinal cord

	Tissue activity	Protein content	Specific activity
Cerebellum	22.4±2.2 (3)	69± 7 (3)	0.33±0.03 (3)
Cerebrum (Telencephalon) (Cerebral hemisphere)	16.4±2.9 (3)	61±10 (3)	0.27±0.00 (3)
Medulla oblongata	17.9±1.8 (3)	75± 5 (3)	0.24±0.02 (3)
Optic lobe	15.5±2.3 (3)	74±12 (3)	0.21±0.00 (3)
Spinal cord	9.3±0.8 (3)	75± 4 (3)	0.12±0.01 (3)

The means±SD are shown with number of samples in parentheses. Tissue activity is expressed as units of glutamine synthetase activity per g tissue. Protein is expressed as mg per g tissue. Specific activity is expressed as units of glutamine synthetase activity per mg protein.