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]

J.T. Webb and G.W. Brown, Jr

Laboratory of Biochemical Ecology, College of Fisheries, University of Washington, Seattle (Washington 98195, USA), 6 November 1979

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.

Concentrations of substrates in the assay mixture were: 60 mM L-glutamine, 15 mM hydroxylamine (NH_2OH), 0.4 mM Na_2ADP, 3 mM $MnCl_2$, 20 mM KH_2AsO_4, and 40 mM imidazole. Incubations were maintained at 25 °C and pH 6.7. Activity was measured by formation of a relatively stable (5–30 min) complex of $FeCl_3$ and γ-glutamyl hydroxamate in acid solution[15] compared with a γ-glutamyl hydroxamate standard at 500 nm. A unit of glutamine synthetase activity is defined as 1 µmole γ-glutamyl hydroxamate produced per min at 25 °C. Protein was determined by the biuret method of Zamenhof[16] with bovine serum albumin as a standard.

The specific activity of each *Squalus acanthias* brain region shows little variation as shown by the standard deviations in column 4 of the table. As observed by others[17–19], brain regions have different, but not a highly regionalized, specific activity of glutamine synthetase. The region of highest activity differs among these reports and may relate to the relative need of the brain regions to detoxify ammonia[8,19]. The relationship of glutamine synthetase to glial content[8,19] of each brain region could also reflect the relative glial content of these regions. As discussed by Martinez-Hernandez et al.[8], this could indicate control by glutamine synthetase of the glutamate pool and regulation of brain excitability. If this is the case, the different excitability of brain regions as expressed by glutamine synthetase activity in *Squalus acanthias* and other animals may be associated with the prominence of each region's function relative to each species' central nervous system adaptations.

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Conversion of mammalian cyclic GMP-dependent protein kinase into modulator-dependent protein kinase (type II) in vitro

W.N. Kuo[1], L.P. Liu, C.F. Duggans, K.M. Foggie, M.J. McNeal and K. Perry

Division of Science and Mathematics, Bethune-Cookman College, Daytona Beach (Florida 32015, USA), 25 October 1979

Summary. The spontaneous conversion of mammalian cyclic GMP-dependent protein kinase (G-PK) into modulator-dependent protein kinase (type II) ($M\text{-}PK_{II}$) in the absence of cGMP or histone was observed in vitro. The findings, together with similarity in substrate protein specificity, suggest that $M\text{-}PK_{II}$ is the catalytic subunit of mammalian G-PK.

Following the initial discovery of G-PK in arthropod tissues[2,3], the cGMP target enzyme was subsequently found in some mammalian tissues[4]. Recently, substantial progress concerning mammalian G-PK has been made, for example: the discovery that crude protein kinase modulator (PKM)[5–8] or purified stimulatory protein kinase modulator (PKM_s)[7,8] is required for maximal G-PK activity under the incubation condition of lower Mg++ concentration; the dissociation of G-PK holoenzyme into its catalytic subunit in the presence of exceedingly high concentration of cGMP or histone[9,10]; and the stimulation of its catalytic subunit in the presence of stimulatory modulator alone[10]. In spite of the above progress, some tasks yet remain. Among them are the conversion of G-PK holoenzyme into its subunits and the reversal of the process in vitro under low concentrations of histone, cGMP, or Mg++; and the stabilization of the subunits in vitro. We now report on the spontaneous conversion of G-PK into $M\text{-}PK_{II}$ in vitro in the absence of cGMP or histone.

Materials and methods. [γ-^{32}P] ATP was purchased from New England Nuclear. cGMP and cAMP were obtained from Boehringer Mannheim (FRG); arginine-rich histone (HA) was obtained from Worthington; another histone (type II-S) and protamine chloride (grade V) were obtained from Sigma. Sephadex G-100 and G-200 were from Pharmacia.

Crude PKM was prepared from liver extracts of 8-month-old ICR mice by boiling and trichloracetic acid-precipitation[5–8]. PKM_s and inhibitory modulator (PKM_I) were partially purified by Sephadex G-100 gel filtration[7,8]. G-PK from guinea-pig fetal lung was purified by Sephadex G-200[12], and the active fractions were pooled, concentrated to about 5 ml (Amicon concentrator using UM-10 membrane). This concentrated G-PK was stored at −20 °C for 8 months