

and terebra. The species *Rhynchochalcis senegalensis* Steffan³ differs from this new species in having very long terebra and in having the preorbital carinae joining the aureular carena clearly.

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ON THE PRESENCE OF CALMODULIN IN THE BRAIN OF CONTROL AND METHYL PARATHION-EXPOSED DEVELOPING TADPOLES OF FROG, *RANA CYANOPHLECTIS*

NAYEEMUNNISA and NAIYARA YASMEEN

Department of Zoology, Bangalore University,
Bangalore 560 056, India.

CALMODULIN, a calcium-binding protein is known to regulate a number of fundamental cellular activities¹. The presence of calmodulin as a component of post-synaptic densities isolated from the rat brain has been reported². It has been suggested that calmodulin may be important in mediating fast axonal transport³. Grab *et al*² and Wood *et al*⁴ found calmodulin to be present on the inner surface of postsynaptic membrane in a variety of nervous system structures. The involvement of calmodulin in the neurotransmitter release has also been demonstrated⁵. However, information is lacking on the presence of brain calmodulin in developing vertebrates during the critical stage of brain development. Hence the present study was undertaken.

Since the critical development period of central nervous system in amphibians occurs during metamorphosis, amphibian tadpoles have been used for this study and the alterations occurring in the regulatory protein, calmodulin, have been analyzed in the brain of control and methyl parathion-exposed animals. Influence of calmodulin extracted from the brain of

methyl parathion exposed animals on the acetylcholinesterase activity and acetylcholine content of control animals has been studied to elucidate the influence of calmodulin from methyl parathion-exposed animals on the neuronal activity of control tadpoles.

Tadpoles of the frog, *Rana cyanophlectis* (20-day-old) in the weight range of 1.5–2 g were obtained from local ponds and acclimated to laboratory conditions for a week, prior to experimentation. The methyl parathion solution (2.5 ppm) in tapwater was prepared and the animals were exposed to this sublethal concentration for 24 hr, after standardization by Probit analysis⁶.

After exposure, the control and the methyl parathion exposed tadpoles were sacrificed and the brain tissue was excised at 0°C. Modulator protein calmodulin was extracted following the procedure of Watterson *et al*⁷ and estimated by the colorimetric method⁸. The total AChE activity and ACh content were estimated by the method of Hestrin⁹.

One ml of 10% homogenate of the brain of the control tadpoles (in phosphate buffer, pH 7) was incubated with 0.1 ml of calmodulin extracted from the brains of control and methyl parathion-exposed animals, respectively for 30 min at 28°C ($\pm 2^\circ\text{C}$). After the incubation period, the samples were analyzed for AChE activity by Hestrin's method⁹. ACh content in the samples was also estimated by Hestrin's method⁹.

The data after statistical analysis are presented in tables 1 and 2. Table 1 shows that calmodulin is present in considerable amounts in the brain of developing tadpoles of the frog, *Rana cyanophlectis*. On exposure to methyl parathion, the brain calmodulin showed a

Table 1 Changes in the level of calmodulin, acetylcholine (ACh) content and activity level of acetylcholinesterase (AChE) in the developing brain of control and methyl parathion-exposed (MPE) tadpoles of frog, *Rana cyanophlectis*.

	C	MPE	
Calmodulin ($\mu\text{mol/kg}$)	14.7 ± 1.0	10.0 ± 0.7	–33.40 $P > 0.05$
Acetylcholine ($\mu\text{mol/g}$)	0.42 ± 0.07	0.97 ± 0.07	+131.90 $P > 0.01$
Acetylcholinesterase ($\mu\text{mol ACh hydro/mg protein/min}$)	1844 ± 67	941 ± 1	–48.95 $P > 0.01$

The values are mean \pm S.D. of 5 observations, + and – sign indicate increase and decrease over controls respectively.

Table 2 Effect of calmodulin extract from the brain of control and methyl parathion-exposed (MPE) tadpoles on the brain acetylcholine content and acetylcholinesterase activity of control developing tadpoles of frog, *Rana cyanophlictis*.

	A	B	C		
Acetylcholine (μ mol/g)	0.350 ± 0.017	0.312 ± 0.012	0.377 ± 0.014	+ 20.64	$P > 0.01$
Acetylcholin- esterase (μ mol/ACh hydro/mg protein/min)	1026 ± 58	1216 ± 57	562 ± 05	- 53.76	$P > 0.01$

A—Control; B—Control after incubation with calmodulin extracted from control tadpoles; C—Control after incubation with calmodulin extracted from MPE tadpoles.

The values are mean \pm S.D. of 5 observations. + and - signs indicate increase and decrease over controls respectively.

significant decrease. The percentage decrease found in the calmodulin, in methyl parathion exposed animals was 33.4.

Table 2 shows that calmodulin from methyl parathion-exposed animals can decrease the hydrolysis of ACh. For instance, calmodulin from methyl parathion-exposed animals brought about 53.76% decrease in AChE activity. Contrary to this, the ACh content in control animals showed 20.64% increase on incubation with the calmodulin extract from methyl parathion-exposed animals. The decrease in AChE was found to be inversely proportional to the increase in ACh content.

Thus the present study demonstrates the presence of calmodulin in considerable amounts in the CNS of the developing tadpoles during the critical stage of brain development. A similar presence of calmodulin in the brain of rat¹⁰ and other tissues¹¹⁻¹⁶ has earlier been reported. Further, Iqbal and Ohs³ suggested that calmodulin may be important in mediating fast axonal transport as it is localized in the neuronal processes.

The calmodulin content in the brain of methyl parathion-exposed animals showed a significant decrease (table 1) and indicates that axonal transport and neurotransmission has been affected in methyl parathion-exposed animals, since calmodulin is known to affect the neurotransmitter release⁶ and has a significant role to play in the junctional transmission of nerve impulse¹. It is possible that on methyl parathion-exposure axonal transport and synaptic transmission is inhibited due to decreased AChE activity.

It appears that the hydrolysis of ACh decreases due to substrate accumulation, as calmodulin extracted

from methyl parathion-exposed animals increased ACh content in the control animals (table 2). Thus the regulatory protein calmodulin extracted from the brain of methyl parathion-exposed tadpoles has some factor or factors capable of decreasing the neuronal activity as evidenced by the decreased AChE activity (table 2).

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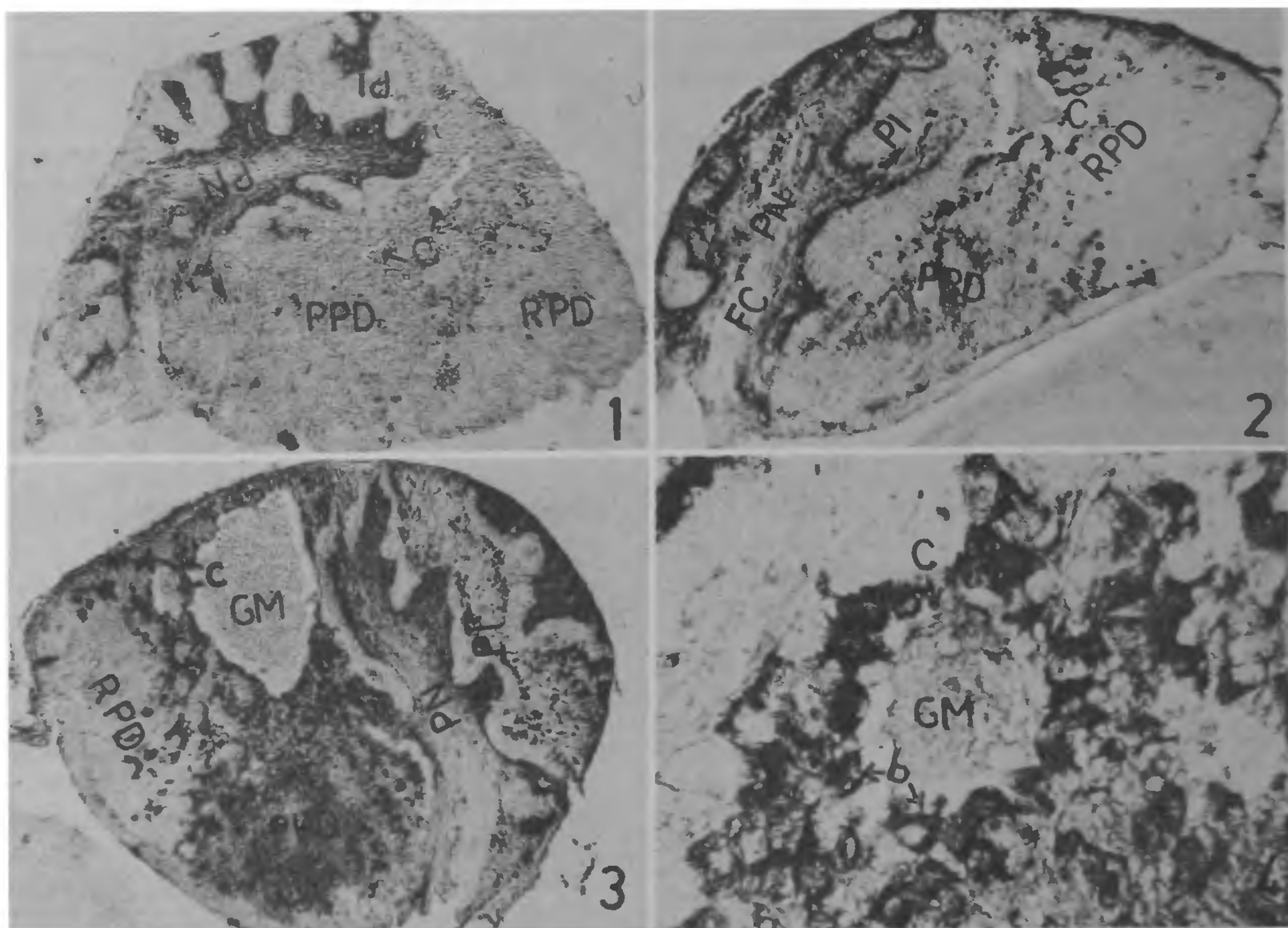
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CAVITIES IN PARS DISTALIS OF PITUITARY GLAND IN *CHANNA PUNCTATUS* (BLOCH)

Y. R. MALHOTRA and K. GUPTA

Department of Biosciences, University of Jammu, Jammu 180001, India.

DOCUMENTATION on the structure of pituitary gland is extensive. But reports on the occurrence of cavities in the gland are scanty. Sahai¹ claimed first report of such cavities in fish during her work on *Ambassis ranga*. But a review of literature suggests that such occurrence was reported in 1942 by Kerr^{2,3} in pars anterior in acipenser, lepidosteus, amia, eel and trout, Tampi⁴ in *Chanos chanos*, Sathyanesan⁵ in *Pangassius pangassius*



Figures 1–4. Longitudinal section of pituitary gland. 1. Smaller cavities (c) during post-spawning period. 2. Large cavity (c) during pre-spawning period and fundibular cavity (FC) 3. Small and large cavity (c) bordered by basophils (b) and granular mass during spawning period. RPD = Rostral pars distalis; PPD = Proximal pars distalis; PI = Pars intermedia; PN = Pars nervosa; GM = Granular mass. 4. Magnified view of cavity (c) showing basophils bordering (b) and granular mass (GM).