P19 DETECTED IN THE RAT RETINA AND PINEAL GLAND IS A
GUANYLYL CYCLASE ACTIVATING PROTEIN (GCAP)

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A reduced retinal tissue concentration of Ca²⁺ ions resulting from illumination of
the photoreceptor cell is the signal for the resynthesis of cGMP by retina-
specific guanylyl cyclases (retGC1 and/or retGC2). This Ca²⁺-dependent
activation of retGCs is mediated by Ca²⁺-binding proteins named GCAPs
(guanylyl cyclase activating proteins) and contributes to the recovery of the
photoreceptor cell to the dark state, thus playing an important role in vision.
Three different GCAPs (GCAP1, GCAP2 and GCAP3) have been identified in
vertebrate retina to date. All activate retGCs at low concentrations of Ca²⁺ (≤50
nM => [Ca²⁺]ₗ), and inhibit the enzyme(s) at high concentrations (≥500 nM =>
[Ca²⁺]ₜ). Although the retina is the primary site where GCAPs are expressed,
GCAP1 was also detected in the bovine pineal gland, and the expression of
genes encoding GCAP1 and GCAP2 was reported for the chicken pineal gland.
The presence of retGC1 in the pineal glands of both species indicates that
synthesis of cGMP in this organ is also regulated in a Ca²⁺-dependent manner
but the physiological significance of this mechanism is unknown.
The aim of our studies was to verify whether the 19 kDa protein (p19), which we
detected in rat retina and pineal gland extracts using an antibody specific to
bovine GCAP1, belongs to the GCAP subfamily. Using an assay of guanylyl
cyclase (GC) activity, we showed that, at [Ca²⁺]ₗ, extracts obtained from the rat
retina and rat pineal gland markedly stimulate the synthesis of cGMP in washed
membranes of bovine rod outer segments (wROS). We also showed that p19,
purified from rat retinal extracts by means of immunoaffinity chromatography,
activates the GCs present in bovine wROS and in the membrane fraction of rat
retinas at [Ca²⁺]ₗ. At the same time, there is no stimulation of GC activity at
[Ca²⁺]ₜ, indicating that the main enzyme in membrane fractions of ROS and rat
retina regulated by the extracts as well as purified p19 is the retGC. Moreover,
purified p19 is recognized by the antibody against bovine GCAP1, and displays
Ca²⁺-dependent changes in electrophoretic mobility, characteristic for all known
GCAPs. All these results clearly show that p19 is a rat guanylyl cyclase-
activating protein (GCAP), most likely GCAP1.