Possible regulation of membrane-associated cyclic AMP phosphodiesterase in rat cerebral cortex by lipids

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Summary. Cyclic AMP phosphodiesterase (PDE) in membrane fraction from rat cerebral cortex was activated by Triton X-100, and treatment at alkaline pH and with phospholipase C. These results suggest that membrane PDE exists in a latent form and is influenced by microenvironmental changes within the membrane. Furthermore, the PDE, unlike soluble enzyme, is not influenced by a protein activator and Ca^{++}.

Cyclic AMP phosphodiesterase (PDE) is present in soluble and particulate fractions in the brain[1,2]. The soluble PDE is activated by a protein activator (PA), which is located in supernatant[3-9] and membrane[10-12] fractions, in the presence of physiological concentration of Ca^{++}. Furthermore, the soluble enzyme is also activated by phospholipids and fatty acids[13,14]. In contrast, it is little known about the properties of the particulate PDE in the brain, although Lindl et al.[15] have recently reported that PDE partially purified from particulate fraction of rat brain is independent from PA and Ca^{++}.

Materials and methods. Male Sprague-Dawley rats, weighing 150–250 g, were used. Rats were decapitated and the cerebral cortexes were homogenized with 0.32 M sucrose containing 5 mM Tris-HCl (pH 7.5). Crude synaptosomes (P-2) and supernatant fractions were prepared according to the method of Whittaker et al.[16]. The P-2 fraction was washed twice with cold sucrose solution and suspended in distilled water. After the hypoosmotic treatment, the mixture was centrifuged at 100,000 × g for 20 min. The resulting pellet was suspended in the homogenizing buffer and used as membrane fraction. Soluble PDE (fraction II) and PA were prepared by Sephadex G-200 gel filtration according to the method of Kakiuchi et al.[8].

The reaction mixture for PDE activity contained 35 mM Tris-HCl (pH 7.5), 2.5 mM $MgCl_2$, 10 µM EGTA, 0.1 µM ^{3}H-cyclic AMP (sp. act. 26 Ci/mmole, Radiochemical Center, Amersham), 0.9 µM cyclic AMP (Sigma Chemical Co) and about 2 µg of enzyme protein in a final volume of 220 µl. This mixture was incubated for 15 min at 30 °C and then 20 µl of snake venom (Sigma Chemical Co, from *Crotalus atrox*, 1 mg/ml) was added to the medium. After a further incubation for 5 min, the reaction was terminated by the addition of 20 µl of 3.5 N HCl. Adenosine and cyclic AMP in the medium were spearated by a paper chromatography (Toyo Filter Paper No. 50, solvent system; water/n-butanol/acetic acid, 5:4:1, v/v) and their contents were measured using a liquid scintilation counter. Protein was determined by the method of Lowry et al.[17].

The membrane fractions were pretreated at various pH and with phospholipase C (Sigma Chemical Co, Type I, from *Clostridium welchii)* as previously reported[18] and centrifuged at 10,000 × g for 20 min. The pellets were washed twice with the homogenizing buffer and suspended in the same buffer for enzyme assay.

Results and discussion. PA and Ca^{++} caused a marked increase of soluble PDE activity, but they did not affect PDE activity in the membrane fractions untreated and treated at various pH and with phopholipase C. Triton X-100 activated membrane PDE at concentrations from 0.02 to 0.5% (w/v) and then caused a slight stimulation of its activity by PA and Ca^{++} (experiment 1). Subsequently we have found that membrane PDE is solubilized by Triton X-100. Gnegy et al.[12] have recently reported that membrane fraction contains much PA. In this experiment, Ca^{++} alone did not activate membrane PDE in the presence of Triton X-100 (data not shown). Furthermore, membrane PDE activity was not influenced by PA and Ca^{++}. Thus, the stimulatory effect of PA and Ca^{++} in the presence of Triton X-100 might be due to the release of soluble PDE contaminated in the membrane fraction, though the fraction was subjected to hypoosmotic treatment.

We have previously shown[18] that the treatment of microsomal suspension at alkaline pH decreases the turbidity of the suspension, possibly indicating the alteration of lipid-protein interaction in the membrane. The incubation of the membrane fraction (200 mg/ml) in the pH range of 7.5–10.0 had little effect on the PDE activity, but above 10.5 there was a large increase in the enzyme activity with a peak effect at pH 11.0 (experiment 2). This result suggests that the PDE is dependent on membrane structure.

Nerve membrane contains much phospholipids and the membrane lipids are likely to play an important role in membrane functions. To elucidate further the nature of membrane PDE, we examined the relation between phospholipids and PDE activity in the membrane using phospholipase C, which hydrolyzed specifically phospholipids. When the membrane fraction (1 mg/ml) was pretreated with phospholipase C (1 mg/ml), the PDE activity increased by about 2fold (experiment 3). Under this condition, phospholipid content in the membrane treated with phospholipase C decreased by about half as much as control. In addition, it is unlikely that the activation is due to soluble end-products of phosphlipase C, since these factors are readily removed by washing of the membrane with sucrose solution following phospholipase C treatment.

Effect of PA and Ca^{++} on soluble and membrane PDE activities of rat cerebral cortex

	Without PA and Ca^{++}	With PA and Ca^{++}
Partially purified soluble PDE	40.6 ± 1.5	79.4 ± 0.8
Membrane PDE		
Experiment 1		
Triton X-100, 0 (w/v) %	4.0 ± 0.4	4.7 ± 0.2
0.01	5.0 ± 0.5	6.6 ± 0.7
0.02	8.9 ± 0.8	12.3 ± 1.0
0.05	8.9 ± 0.9	12.3 ± 1.1
0.10	10.0 ± 0.8	12.4 ± 0.9
0.5	9.8 ± 0.9	11.5 ± 0.7
Experiment 2		
Treated at pH 7.5	3.3 ± 0.4	3.7 ± 0.3
10.0	3.9 ± 0.5	4.5 ± 0.4
10.5	5.0 ± 0.9	6.0 ± 0.3
11.0	7.9 ± 1.2	7.7 ± 0.5
11.5	1.9 ± 0.3	2.9 ± 0.9
Experiment 3		
Control	3.8 ± 0.1	4.8 ± 0.4
Phospholipase C-treatment	6.1 ± 0.9	6.2 ± 0.7

Activity is expressed as nmoles cyclic AMP hydrolyzed/mg protein/20 min. After the treatment at various pH (experiment 2) and with phospholipase C (experiment 3), membrane fractions were washed twice with the homogenizing buffer. Ca^{++} (as $CaCl_2$) and PA were used at concentrations of 0.1 mM and 2.6 µg, respectively. Each value is the mean±SE (n = 3-4).

Thus, membrane PDE is activated by a brekadown of the membrane phospholipids.

Recently Lindl et al.[15] showed that the particulate PDE was not activated by PA and Ca^{++}. Our present result agrees with their observation. Now, the possible question arises that this insensitivity of membrane PDE to PA and Ca^{++} may be accounted for by the presence of sturable PA and Ca^{++} in the membrane fraction. In preliminary experiment, we solubilized the PDE with Triton X-100 from the membrane fraction and chromatographed it on Sephadex G-200 in the presence of EGTA to separate PDE from PA in the membrane. However, we failed to detect any PDE from the membranes which is activated by PA and Ca^{++}. These results suggest that membrane PDE, unlike soluble enzyme, does not react on PA and Ca^{++}. Though it is obscure whether the membrane-bound and soluble activities stem from the same enyzme protein, the present study indicates that the nature of the membrane PDE is different from that of the soluble one.

Although we used cyclic AMP as substrate of PDE in this work, studies on the properties of membrane PDE in which cyclic GMP is used as the substrate, are also required.

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Hybridation en laboratoire de *Mus musculus* L. et *Mus spretus* Lataste
Hybridization between *Mus musculus* L. and *Mus spretus* Lataste under laboratory conditions

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Summary. 2 species of mice *Mus musculus brevirostris* and *Mus spretus* are sympatric in Southern France and reproductively isolated. However, under laboratory conditions, they breed and give fully viable F1 hybrids. This indicates that premating isolating mechanisms probably occur in nature and have yet to be investigated. Moreover, it now enables us to introduce new genetical markers in laboratory strains.

Les techniques d'électrophorèse ont permis de montrer que la souris à queue courte de Méditerranée occidentale, jusqu'alors désignée comme *Mus musculus spretus*, est en fait génétiquement isolée de la sous-espèce *Mus musculus brevirostris* avec laquelle elle se trouve en sympathie. Si l'on s'en tient au concept biologique de l'espèce, elle constitue une espèce à part entière et doit être désignée par le binomen *Mus spretus* Lataste[2,3].

Tandis que *M. spretus* est strictement sauvage, sans colonies établies à l'intérieur des maisons, *M. m. brevirostris* est surtout commensale, mais il en existe également des colonies permanentes à l'extérieur. Dans beaucoup d'endroits, les populations s'interpénètrent, et la question se pose de savoir quelle est la nature de la barrière reproductive entre les 2 espèces.

Pour ces raisons, et également parce que *M. spretus* possède plusieurs variants biochimiques nouveaux[4] qu'il serait très intéressant d'introduire dans des souches de laboratoire, nous avons entrepris de les croiser en animalerie.

Matériel et méthode. Les individus utilisés pour nos croisements appartenaient soit à des populations de *M. spretus* et *M. m. brevirostris* capturés dans les environs de Montpellier, soit à la souche consanguine BALB/c. Les animaux étaient placés deux par deux dans des conditions standard d'élevage: cages plastiques Iffacredo (14×18×25 cm), nourriture Labena, température 21 °C, photopériode 16 h.

Résultats. Hybrides de F1. Nous avons obtenu 20 descendants de F1, 7♂ et 13♀, provenant des couples suivants: ♂ *M. spretus* n° 1×♀ BALB/c n° 1, ♂ *M. spretus* n° 2×♀ BALB/c n° 2, ♂ M. spretus n° 3×♀ *M. musculus brevirostris* n° 1.

Aucun des couples hétérospécifiques où la femelle était *M. spretus* n'a donné de descendance. De même, des couples témoins *M. spretus* × *M. spretus* ne se sont pas reproduits, ceci suggérant que les femelles *M. spretus* sont apparemment incapables de se reproduire dans les conditions d'élevage de notre laboratoire. Les hybrides de F1 sont vigoureux et se développent normalement. Il semblerait que le sex-ratio soit en faveur des femelles dans la descendance hybride, mais cette remarque demande à être confirmée sur un échantillon plus grand.

Croisement-retours (back-cross). Toutes les femelles de F1 qui ont été croisées soit avec des mâles BALB/c, soit avec des mâles *M. m. brevirostris*, se sont révélées fertiles, donnant naissance à une abondante progéniture d'individus sains et normaux. Par contre, aucun croisement-retour avec