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Received 2 April 1991

Action of uroporphyrinogen decarboxylase on uroporphyrinogen III

One of the outstanding questions concerning the enzymic decarboxylation of uroporphyrinogen III is whether the reaction is clockwise and orderly starting from the ring-D acetate group or is random. Evidence in support of a clockwise mechanism was provided by the fact that the intermediates isolated from the faeces of hexachlorobenzene-poisoned rats were 7d, 6da and 5dab, respectively (Jackson *et al.*, 1976). We have also confirmed this by h.p.l.c. analysis. On the other hand, all 14 intermediates were detected in normal human urine (Lim *et al.*, 1983) and incubation of uroporphyrinogen III with red cell haemolysates produced a mixture of isomers (Lash, 1979; Luo & Lim, 1990), indicating random decarboxylation.

Lash (1991) postulates that the uroporphyrinogen III used in the enzyme reaction may be presented randomly to the enzyme leading to random decarboxylation while with PBG as substrate the enzymically produced uroporphyrinogen III is 'handed on' to uroporphyrinogen decarboxylase in a specific orientation, resulting in specific decarboxylation of the ring-D acetate group. We accept this explanation is plausible and agree that it should be tested experimentally.

Whether the preferential accumulation of 7d in PCT and in hexachlorobenzene-poisoned rats is a result of damage to uro-

porphyrinogen decarboxylase, however, remains an open question.

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Received 8 July 1991

Inhibition of adenylate cyclase by Ca^{2+} — a counterpart to stimulation by Ca^{2+} /calmodulin

Stimulation of adenylate cyclase, mediated by Ca^{2+} acting via calmodulin, is the longest established example of potential positive 'crosstalk' between the Ca^{2+} and cyclic AMP-signalling systems [1]. Ca^{2+} , in concentrations that are achieved intracellularly, elicits a prominent stimulation of adenylate cyclase, which is variable in magnitude, depending on the tissue [2]. It is assumed that neurotransmitters that elevate $[\text{Ca}^{2+}]_i$ should also elevate cyclic AMP by this mechanism in intact neurons. Whether this actually occurs in cells has never been proven, although a considerable number of reports indicate that $[\text{Ca}^{2+}]_i$ -mobilizing neurotransmitters elevate cyclic AMP in brain slice preparations [3–5]. It has also been proposed that such Ca^{2+} /calmodulin-stimulated adenylate cyclase is the form that is susceptible to neurotransmitter inhibition [2,6,7] and that this inhibition is a significant component of presynaptic autoinhibitory mechanisms [8]. Ca^{2+} /calmodulin-stimulated adenylate cyclase was first described in brain [9,10] and partially purified from this source. Development of antibodies against such preparations suggested a rather limited tissue distribution [11,12]. Further purification, leading to the recent cloning and expression of a Ca^{2+} /calmodulin-stimulated adenylate cyclase [13] has confirmed the largely neuronal distribution of the mRNA encoding this species [14].

In contrast to the stimulatory effect of Ca^{2+} /calmodulin on brain adenylate cyclase, a growing number of reports indicate that low concentrations of Ca^{2+} inhibit adenylate cyclase in plasma membranes from other sources. Adenylate cyclase from pituitary tissue [15], purified somatotrophs [16], GH3 cells [17], platelets, NCB-20 cells [18] and cardiac sarcolemma [19] is inhibited by 35–50% by Ca^{2+} in the same concentration range that stimulates brain adenylate cyclase. The magnitude of inhibition is greater than that elicited by G_i -protein-coupled receptors and is additive with their effects [15–19]. (Inhibition by Ca^{2+} in the low micromolar range is not to be confused with inhibition by Ca^{2+} in the submillimolar range. This latter inhibition is displayed by all adenylate cyclases regardless of their source and of their response to Ca^{2+} in the submicromolar range, including, for instance, the enzymes from brain, liver and cardiac tissue, which are stimulated, unaffected and inhibited, respectively, by submicromolar $[\text{Ca}^{2+}]$. In a system in which inhibition by Ca^{2+} is encountered both in the submicro- and submillimolar range, the two inhibitory effects are generally separated by a

plateau in activity between approximately 1 and 10 μM (cf. [18]). This low potency inhibition by Ca^{2+} has been postulated to reflect competition between Ca^{2+} and Mg^{2+} at an allosteric site on the catalytic unit of the cyclase [20,21].

It may turn out that the inhibition by Ca^{2+} of adenylate cyclase is a widespread form of regulation. However, the effect may be overlooked since (i) it is mediated by concentrations of Ca^{2+} that are present as a background in many adenylate cyclase assays and (ii) the inhibition does not preclude the detection *in vitro* of either stimulation or inhibition by hormones.

The Ca^{2+} inhibition is not mediated nor affected by calmodulin. Indeed, it does not appear to be mediated by any readily dissociable factor. The inhibition is not modulated by pertussis toxin, phosphodiesterase or protein kinase C in membranes, although stimulated activity states are the most susceptible to inhibition [15–19]. Therefore Ca^{2+} inhibition may be due to a direct action on the adenylate cyclase catalytic subunit. Thus, it may be anticipated that the cloning and expression of adenylate cyclase from one of the sources that expresses Ca^{2+} inhibition will reveal a novel enzyme. The current availability of four cloned adenylate cyclase species [13,22,23] suggests that this hypothesis will soon be tested.

From a regulatory perspective, the inhibition of adenylate cyclase by Ca^{2+} is potentially of major physiological significance, in that it offers the possibility that Ca^{2+} -mobilizing systems can exert a negative influence on cyclic AMP generation as a result of direct effects of Ca^{2+} at the catalytic unit of adenylate cyclase in the intact cell. This supposition is not simple to evaluate in intact cells for a number of reasons. First, there are a number of possible mechanisms whereby Ca^{2+} might regulate adenylate cyclase; for instance, various Ca^{2+} -dependent protein kinases and phosphodiesterases inhibit cyclic AMP production in a number of systems [24–26]. Second, hormonal elevation of $[\text{Ca}^{2+}]_i$ cannot readily be achieved without other effects; for instance, the activation of protein kinase C as a result of the production of diacylglycerol from $\text{PtdIns}(4,5)\text{P}_2$. Careful experiments are needed to prove that elevated $[\text{Ca}^{2+}]_i$ can be a primary cause of inhibition of cyclic AMP accumulation, as distinct from other mechanisms that can be associated with elevation of $[\text{Ca}^{2+}]_i$, such as Ca^{2+} /calmodulin-dependent kinases, protein kinase C, Ca^{2+} /calmodulin-stimulated cyclic AMP phosphodiesterase, etc. Useful tools for this purpose are thapsigargin or ionomycin. Both of these compounds, in suitable doses and by distinct mechanisms, elevate $[\text{Ca}^{2+}]_i$ without activating protein kinase C [27]. An additional strategy would be to use cell permeant Ca^{2+} -chelators, such as the acetomethoxy ester of BAPTA; intracellular BAPTA could selectively prevent the $[\text{Ca}^{2+}]_i$ rise associated with hormonal stimulation, without perturbing other hormone effects.

There is some supporting evidence for a physiological role for Ca^{2+} inhibition of adenylate cyclase from studies performed in intact cells. In purified somatotrophs, reduction of $[\text{Ca}^{2+}]_i$, either by incubation in Ca^{2+} -free medium or in the presence of Ca^{2+} -channel blockers, results in an increase in GRH-stimulated cyclic AMP accumulation [16]. In NCB-20 cells, bradykinin, which elevates $[\text{Ca}^{2+}]_i$ transiently, as a consequence of $\text{PtdIns}(4,5)\text{P}_2$ hydrolysis, causes a transient inhibition of cyclic AMP accumulation. In addition, blockade of the bradykinin-induced Ca^{2+} -rise by BAPTA reduces the bradykinin-dependent inhibition of cyclic AMP accumulation [18]. Further experiments are required to evaluate the extent of inhibition of cyclic AMP accumulation that is caused by $[\text{Ca}^{2+}]_i$, as distinct from other processes that are also activated by Ca^{2+} -mobilizing hormones.

The physiological significance of this inhibition has been suggested in a few instances, e.g. somatotrophs and cardiac tissue. In anterior pituitary cells, $[\text{Ca}^{2+}]_i$ can be elevated as a result of stimulation of cyclic AMP accumulation. In somato-

trophs, it has been proposed that the GRH-induced elevation in $[\text{Ca}^{2+}]_i$ (mediated by cyclic AMP) serves not only to trigger GH release, but also to inhibit cyclic AMP formation, thereby attenuating the secretory response [16]. In cardiac sarcolemma, $[\text{Ca}^{2+}]_i$ can be elevated by increasing cyclic AMP production, as a result of β -adrenergic stimulation; the inhibition of adenylate cyclase caused by the elevated $[\text{Ca}^{2+}]_i$ provides a negative feedback, which dampens the Ca^{2+} -transient [19]. It may also be speculated that selective localization of Ca^{2+} -inhibitable adenylate cyclase within cells could provide discrete local consequences for the elevation of $[\text{Ca}^{2+}]_i$ on cyclic AMP accumulation.

It seems likely that further instances of Ca^{2+} inhibition of adenylate cyclase will be discovered and the contribution of this novel mode of interaction between the Ca^{2+} -elevating system and cyclic AMP accumulation will be evaluated in terms of the overall interaction between the two systems at the cellular level. For the present, it adds another strand in the intricate web of cellular interactions that comprise hormonal regulation [15].

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Received 17 May 1991