GUANYL CYCLASE IN RAT BRAIN SUBCELLULAR FRACTIONS

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1. Introduction

Guanyl cyclase, the enzyme which catalyzes the formation of guanosine cyclic 3′,5′-monophosphate (cyclic GMP) from GTP, has been detected in all mammalian tissues studied. Guanyl cyclase activities are high in lung [1–3] and especially high in purified bovine retinal outer segments [4]. Brain has a lesser, but nevertheless appreciable, guanyl cyclase activity. We have determined the guanyl cyclase activity in purified fractions of defined neuronal or synaptosomal origin, in order to throw some light upon possible roles for guanyl cyclase and cyclic GMP in brain.

2. Methods

Subcellular fractions were prepared from adult rat brain by methods described previously [5]. The post-mitochondrial supernatant was centrifuged at 78 000 $g \times 120$ min to give the *primary soluble* and the *microsomal* fractions. Synaptosomes were sub-fractionated after osmotic shock to give *synaptosomal plasma membranes* [5], *synaptic vesicles* [6] and *synaptosomal soluble proteins*. The latter soluble fraction was only slightly contaminated with non-synaptosomal soluble proteins since three washes of the crude mitochondrial fraction have been shown to eliminate all the non-occluded lactate dehydrogenase (LDH) [5]. Due to the low protein concentrations the synaptosomal soluble fraction had to be concentrated (8–10-fold) by ultrafiltration on PM10 Amicon filters.

Guanyl cyclase activity was determined using [^{32}P]α-GTP as substrate (0.185 mM, 0.5–1 $\times 10^6$ cpm) exactly as previously described [4]. Preliminary experiments showed that the Mn^{2+} concentration used (7.7 mM) was at, or near, the optimum concentration for all fractions. Cyclic GMP (5.75 mM) was added to the incubation mixture to reduce degradation of [^{32}P]cyclic GMP by phosphodiesterases, and the extent of the degradation was checked routinely by addition of [^{3}H]cyclic GMP. All fractions were appropriately diluted to keep hydrolysis of cyclic GMP during the incubation period below 10%.

LDH was assayed by following the oxidation of NADH in the presence of pyruvate at 340 nm [7] and

Table 1

Guanyl cyclase and lactate dehydrogenase activities in rat brain subcellular fractions.

Fraction	Guanyl cyclase		Lactate dehydrogenase	
	Activity[a]	Enrichment[b]	Activity[c]	Enrichment[b]
Homogenate	31 ± 3.3	1	42	1
Primary soluble	69 ± 16[d]	2.2	145	3.5
Synaptosomal soluble	239 ± 58	7.7	85	2.1
Synaptosomal plasma membranes	33 ± 7.6	1.1	0.3	0.007
Synaptic vesicles	12 ± 2.9	0.4	0.8	0.01
Microsomes	24 ± 3.9	0.8	21	0.5

[a] Guanyl cyclase activity: pmol/min/mg protein expressed as the mean ± S.E.M. for five experiments.
[b] Enrichment: specific activity of fraction/specific activity of homogenate.
[c] Lactate dehydrogenase activity: μmole/h/mg protein.
[d] $P < 0.01$ if compared with synaptosomal soluble fraction.

Table 2
Guanyl cyclase activity in rat brain soluble fractions prepared under isotonic or hypotonic conditions.

	Activity (pmol/min/mg protein)
Isotonic	60
Hypotonic	68

Two rat brains were homogenized in either sucrose (320 mM)–phosphate (1 mM)–EDTA (1 mM) or in phosphate (1 mM)–EDTA (1 mM). The crude homogenates were centrifuged 1000 g × 15 min, the pellet obtained washed once with the buffer used for homogenization and the combined supernatants centrifuged 78 000 g × 120 min to give the soluble fractions.

proteins were estimated by the technique of Lowry et al. [8] after precipitation with 1 M $HClO_4$.

3. Results

In accord with previous studies on the subcellular distribution of guanyl cyclase activity in lung and liver [1, 2] the highe it specific activities were found in the soluble fractions. But, whereas the specific activity of LDH was highest in the primary soluble fraction (table 1), guanyl cyclase was over three times more active in the synaptosomal soluble fraction than in the primary soluble fraction. Since the higher guanyl cyclase activity in the synaptosomal soluble fraction could have been due to the liberation of membrane-bound guanyl cyclase by the osmotic shock applied to the synaptosomes, primary soluble fractions were prepared under hypotonic and isotonic conditions (table 2). There was a slight increase in the specific activity of the fraction prepared under hypotonic conditions, probably due to the release of synaptosomal guanyl cyclase-rich soluble proteins. However since LDH activity was almost undetectable in the synaptosomal plasma membranes, whereas the guanyl cyclase activity was equal to that of the starting homogenate, we cannot exclude the possibility of a specifically synaptosomal, membrane-bound, guanyl cyclase.

Since the synaptosomal soluble fraction had to be concentrated by ultrafiltration, the influence of this treatment on two other fractions, the primary soluble fraction and the synaptic vesicles, was studied. No differences of the guanyl cyclase specific activities were observed before and after concentration.

4. Discussion

The results reported here show that rat brain guanyl cyclase is concentrated in soluble fractions, as is the case in lung and liver [1, 2]. But in brain tissue, two soluble compartments can be resolved: the primary soluble fraction, i.e. a postmicrosomal supernatant derived in an analogous manner to the soluble fraction of other tissues, and the soluble compartment of the synaptosomes. The synaptosomal components sediment first with the crude mitochondrial pellet and then with the synaptosomes, but can be released into the supernatant by osmotic shock of these particles [9, 10]. The synaptosomal soluble fraction had the highest guanyl cyclase activity of the fractions studied. By contrast, LDH had a higher activity in the primary soluble fraction. The distribution of guanyl cyclase thus differed from that of general soluble markers such as LDH, and that reported for free amino acids [11] and 5-hydroxytryptophan decarboxylase [9]. It also differed from the distribution of two glial soluble markers, S-100 protein and butyryl cholinesterase [12], but resembled the distribution of neuronal soluble markers, glutamate decarboxylase [13] and tyrosine hydroxylase [14]. Guanyl cyclase would thus seem to be enriched in the cytoplasm sequestered within the synaptosomes as are these two enzymes involved in neurotransmitter metabolism.

Two explanations could be given for the enrichment of a soluble component within the synaptosomes. Either this component occurs predominantly in neurons and is therefore diluted in the primary soluble fraction by the cytoplasm derived from glial cells, or it is concentrated within the neuron at the level of the presynaptic nerve terminal. We tend to favour the former possibility in the case of guanyl cyclase based on a study of the development of guanyl cyclase in cultures of cortical cells, where guanyl cyclase activities are higher in neuron-enriched, than in glial cell-enriched cultures [15].

Guanyl cyclase shows considerably higher relative specific activities than LDH not only in the synaptosomal soluble fraction, but also in the synaptosomal plas-

ma membranes. Considering the possible role of cyclic GMP in synaptic transmission [16], such a localization could be of considerable importance. Work is now in progress to investigate whether the guanyl cyclase found associated with the synaptosomal plasma membranes represents soluble enzyme adsorbed to or sequestered within these membranes, or whether it represents an intrinsic synaptosomal plasma membrane activity.

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