VIEWPOINT

Enhancement of acetylcholinesterase synthesis by glycyl-L-glutamine: an example of a small peptide that regulates differential transcription?

George B. Koelle

Glycyl-L-glutamine has been shown to maintain the acetylcholinesterase content of the preganglionically denervated superior cervical ganglion of the cat. It acts at a stage prior to the aggregation of the monomeric G_1 form of the enzyme to higher polymers. George Koelle *proposes that it may do so by regulating the transcription of DNA to mRNA, in a manner analogous to that of triiodothyronine. Other small peptides may function similarly.*

Triiodothyronine (T_3; Fig. 1) and its peripheral precursor thyroxine (T_4) appear to exert most of their widespread metabolic effects by regulating the transcription of specific genes to mRNAs that encode several enzymes and other proteins[1,2]. Our studies of the action of glycyl-L-glutamine (GlyGln; Fig. 1) in maintaining the acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BuChE; EC 3.1.1.8) contents of the preganglionically denervated superior cervical ganglion of the cat suggest that this dipeptide may act in a more restricted manner by a similar mechanism[3–10]. It is possible that similar small peptides may likewise be responsible for regulating the synthesis of other proteins.

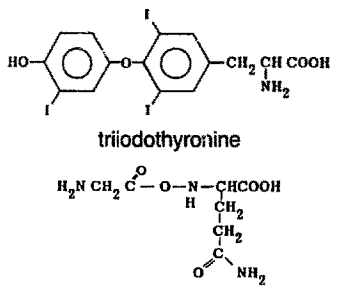

Fig. 1. Structures of triiodothyronine and glycyl-L-glutamine.

AChE distribution

It was reported in 1945 by Sawyer and Hollinshead[11] and later confirmed[3] that preganglionic denervation of the cat superior cervical ganglion results within a few days in a permanent loss of over 80% of its AChE and 40% of its BuChE content. Light microscopic histochemical studies showed that in the normal superior cervical ganglion and stellate ganglion, AChE is present in high concentration in the neuropil (the intertwined preganglionic fibers, their terminals, and the dendrites of the ganglion cells) and in the perikarya of scattered cholinergic ganglion cells, and in traces in the perikarya of the greatly predominant adrenergic ganglion cells. Following preganglionic denervation, the enzyme disappears from the neuropil but remains in the ganglion cell perikarya[12] (Fig. 2). These observations led to the conclusion, eventually modified, that in the normal superior cervical and stellate ganglion the AChE of the neuropil is confined to the preganglionic fibers and their terminals[13]. Subsequent examination of the superior cervical ganglion (in which the ganglion cells are nearly exclusively adrenergic) by electron microscopic histochemistry revealed that in the normal superior cervical ganglion AChE is indeed distributed evenly throughout the lengths of the cholinergic preganglionic fibers and their terminals; however, it is present also at the membranes of both the dendrites and perikarya of the ganglion cells[14]. The demonstration that denervation is followed by the total disappearance of AChE from both pre- and postsynaptic membranous sites[15] (Fig. 3) confirms the earlier light microscopic observations.

These findings raised the question of why the integrity of the presynaptic fibers is essential for the maintenance of the enzyme at postsynaptic membranes. One possibility is that under normal conditions AChE is released continuously by the former[16,17] and migrates through the synaptic cleft to become aligned on the postsynaptic membranes. This explanation seems unlikely for two reasons: the even distribution of AChE at postsynaptic membranous sites remote from synapses; and the absence of detectable AChE in the synaptic clefts[14]. An alternative explanation is that the preganglionic fibers release a neurotrophic factor that is essential for the synthesis of AChE by the rough endoplasmic reticulum of the ganglion cells, for subsequent export to their perikaryonal and dendritic membranes.

A neurotrophic factor?

This latter possibility was tested as follows. Cats were anesthetized with ketamine and both cervical sympathetic trunks were sectioned. The following day they were reanesthetized with sodium pentobarbital, artificially respired, and the external carotid and lingual branches of the common carotid arteries were ligated bilaterally to divert most of the bloodflow to the superior cervical ganglia via the internal carotid and occipital arteries[18]. An infusion needle was inserted into the right common carotid artery, through which an

George Koelle is Distinguished Professor in the Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104–6084, USA.

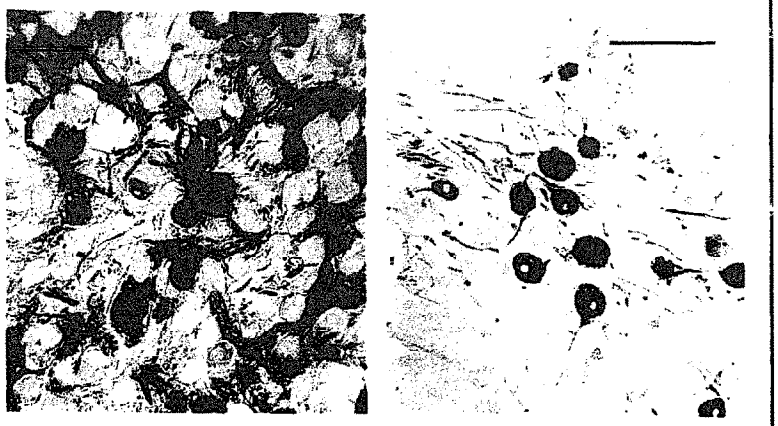

Fig. 2. Light micrographs of cat stellate ganglion stained for AChE by the copper thiocholine method. Scale bar = 0.1 mm. **Left,** *normal;* **right,** *preganglionically denervated.* (Taken from Ref. 12 with permission.)

aqueous extract of a cat brain, spinal cord and sciatic nerves was infused continuously for 24 hours under constant surveillance. Repeated doses of sodium pentobarbital were given to maintain deep anesthesia, and mephentermine or atropine was given as required. Exactly 48 hours postdenervation the cats were sacrificed by an overdose of anesthetic, and both superior cervical ganglia were excised, homogenized and assayed for AChE, BuChE and protein contents. The superior cervical ganglia of cats so treated maintained nearly the same content of AChE as was present 24 hours postdenervation (approximately 85% of normal) in comparison with similarly treated saline-infused controls in which the AChE contents fell to approximately 50%. The BuChE content was also partially preserved by the infusion but less strikingly[3,5].

Similar infusions of acetylcholine, nerve growth factor (2.5S and 7S) or extracts of liver or skeletal muscle were ineffective. cAMP and extracts of gut showed borderline activity. When the original brain/cord/nerve extract was dialysed, the retentate was inactive but dialysates at molecular weight cut-off 1000 showed full activity. This was lost following incubation with carboxypeptidase A type I; controls incubated with bovine serum albumin and also heated subsequently retained activity[4]. These results indicated that the neurotropic factor is a small, heat-stable peptide.

Glycyl-L-glutamine

Following a report by Haynes and Smith[19] that glycyl-L-glutamine (GlyGln), the terminal cleavage dipeptide of β-endorphin that occurs in brain[20], induces the formation of higher polymers of AChE in cultured embryonic rat and chick skeletal muscle, we tested this compound in the cat preparation. Surprisingly, GlyGln had no effect on the AChE content of the directly infused denervated right superior cervical ganglion but maintained the enzyme in the circulatory remote left superior cervical ganglion, where the infused material arrives via the anastomoses of the right and left internal carotid and occipital arteries. This suggested that a metabolite of GlyGln, formed in the circulation, is the active neurotrophic factor. Infusions of glycine and glutamine were ineffective; glycyl-L-glutamic acid and glutamic acid were effective at both superior cervical ganglia but less so than GlyGln at the left superior cervical ganglion only[5,6]. Aspartic acid, GABA and pyroglutamic acid were also inactive[6].

More recently we have tested an alternative explanation for the restricted site of action of GlyGln: that it must combine relatively slowly with a component of plasma to allow its penetration to the cytoplasm of the ganglion cells. When GlyGln was incubated at 5°C overnight with fresh or heat-treated (60 min at 60°C) cat plasma then diluted with 0.9% NaCl prior to infusion, it exhibited full AChE-maintaining activity in both right and left superior cervical ganglia. [No attempt was made to identify the effective plasma component(s) other than to demonstrate that it was present in both dialysate and retentate at a molecular weight cut-off of 10 000.] A maximal effect was obtained with infusion of a 3×10^{-6} M solution of GlyGln; at concentrations of 10^{-4} M or higher the effect was inhibitory[7].

GlyGln thus appears to be a potent, directly acting neurotrophic factor for the maintenance of AChE in the denervated cat superior cervical ganglion. It is not known whether it is identical with the endogenous factor of the

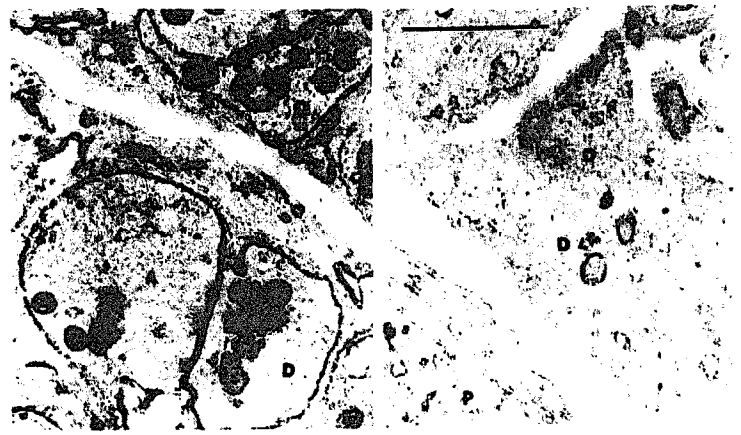

Fig. 3. Electron micrographs of cat superior cervical ganglion stained for AChE by the bis(thioacetoxy)aurate method. Scale bar = 1 μm. **Left,** *normal;* **right,** *preganglionically denervated.* **A,** *axon;* **D,** *dendrite.* (Taken from Refs 14 and 15 with permission.)

The molecular forms of acetylcholinesterase

The superior cervical ganglia of the rat and steer have been shown to contain six major molecular forms of AChE: the globular monomer (G_1), dimer (G_2), and tetramer (G_4), and the collagen-tailed asymmetric A_4, A_8 and A_{12} forms (Fig.). The predominant forms are: G_1, located in the cytoplasm; G_4, at membranous sites; and A_{12}, which is probably confined to synaptic sites[1]. The G_1 form is synthesized initially then converted sequentially to the higher polymers[2]. However, only a fraction of the G_1 is converted to G_4 (Ref. 3) and probably only a fraction of G_4 is converted to A_{12}; the remainder is degraded.

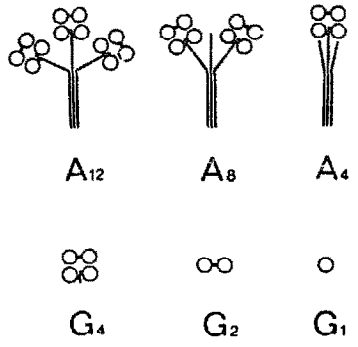

Fig. The molecular aggregate forms of acetylcholinesterase, as established in Electrophorus *and other species. Globular monomer* (G_1), *dimer* (G_2) *and tetramer* (G_4), *and asymmetric, collagen-tailed* A_4, A_8 *and* A_{12}. (Taken from Ref. 1 with permission.)

References

1 Massoulié, J. and Bon, S. (1982) *Annu. Rev. Neurosci.* 5, 57–106
2 Brockman, S. K., Usiak, M. F. and Younkin, S. G. (1986) *J. Biol. Chem.* 261, 1201–1207
3 Lazar, M., Salmeron, E., Vigny, M. and Massoulié, J. (1984) *J. Biol. Chem.* 259, 3703–3713

dialysed extract, or with the hypothetical factor that is presumed to be released by the preganglionic fibers of the superior cervical ganglion.

GlyGln acts at early stage in AChE synthesis

Recently in the laboratory of Jean Massoulié at the Ecole Normale Supérieure, Paris, we investigated the stage at which GlyGln acts, by determining its effects on the various molecular aggregate forms of AChE (see Box) as distinguished by sucrose density gradient centrifugation. In the cat the dominant peak was found to be the membrane-located G_4; considerably lower peaks were found for the cytoplasmic (or endoplasmic reticular) G_2 and G_1 forms, and there was only a trace of the synaptic A_{12} peak. Four days postdenervation there was a marked fall in the G_4 peak, and only slight falls in the others[8] (Fig. 4).

In another series of experiments, ganglia were excised one to five days postdenervation, sectioned at 400 µm and cultured for 24 or 48 hours. In some experiments, AChE was inactivated irreversibly by treatment with (2-mercaptoethyl)-dimethylamine acid oxalate (217 AO) prior to culture. Sections were then homogenized in AChE-extraction medium, and fractionated following sucrose density gradient centrifugation. There was a marked progressive fall in the total and newly synthesized AChE contents of cultured sections excised from one to five days postdenervation. In essentially all cases the chief effect of 10^{-5} M GlyGln was enhancement of the G_1 peak, with progressively lesser enhancement of the G_2 and G_4 peaks; the A_{12} peak was too small to permit assessment. At higher concentrations, as in the *in-vivo* experiments, the effect was inhibitory[9].

The major finding of these studies has now been confirmed by infusion experiments *in vivo*[10]. Thus GlyGln appears to act at an earlier stage of AChE synthesis (transcription, translation, conversion of inactive precursor) rather than by regulating the aggregation of the monomer to higher polymers, as was hypothesized earlier[6,19].

As noted above, the maximal effect of GlyGln *in vivo* was obtained with infusions of 3×10^{-6} M solutions that had been treated with plasma. Since the infused solution was diluted at least 30-fold in the bloodstream before reaching the superior cervical ganglion, it may be assumed that the effective concentration at the ganglion cells is less than 10^{-7} M. While this is considerably lower than the optimal concentration found *in vitro*, it is probably a better index of efficacy than that obtained under the less physiological conditions in culture. With this degree of potency, it seems likely that GlyGln acts by combining with a specific receptor.

Most of the recognized neuronal receptors are located at the external surface of the effector cells, where they are coupled either with an ion gate or with internally located second messengers such as cAMP. However, as noted in the introduction, the receptors for the thyroid hormones, like the steroid receptors, are intracellular; they are probably present on the genes, where they are believed to regulate the transcription of DNA to mRNA for export to the cytoplasm and the eventual determination of protein synthesis[1].

GlyGln, like the thyroid hormones, appears to combine with certain constituents of plasma to

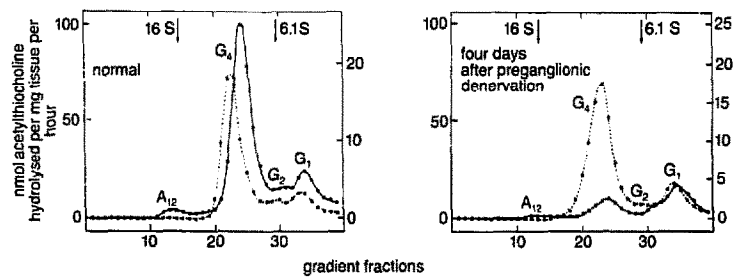

Fig. 4. Sedimentation profiles of AChE (**unbroken line**) *and BuChE* (**broken line**) *of the cat superior cervical ganglion. Arrows indicate the positions of the markers, alkaline phosphatase (6.1S) and β-galactosidase (16S).* (Taken from Ref. 8 with permission.)

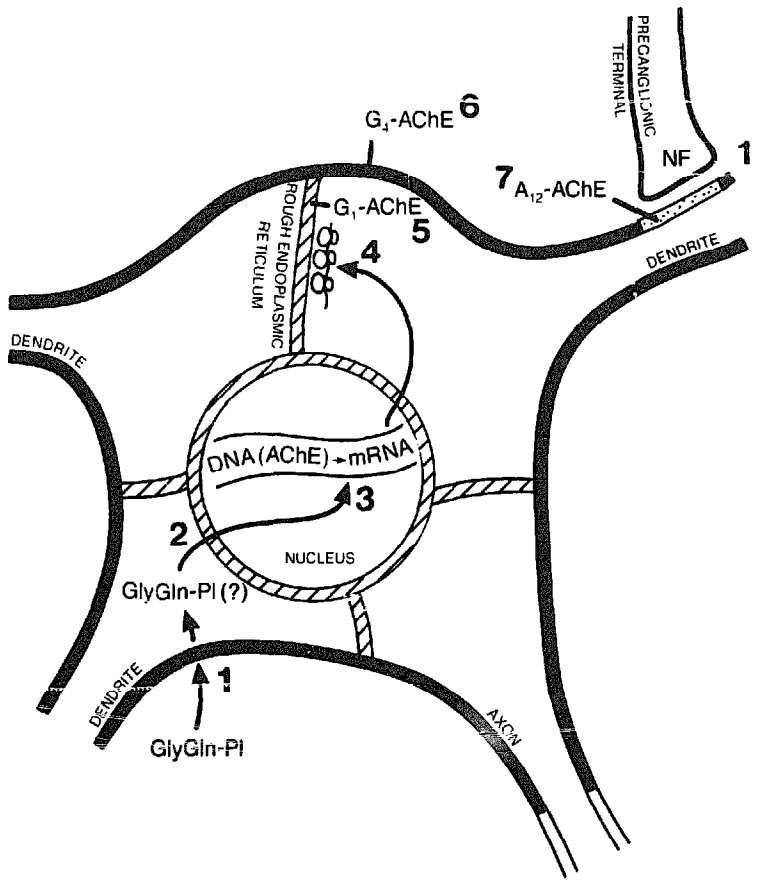

Fig. 5. Working hypothesis for regulation of AChE synthesis by GlyGln in an adrenergic neuron of the cat superior cervical ganglion. **(1)** *GlyGln, combined with plasma factor* **(PI)**, *or a neurotrophic factor* **(NF)** *released by the preganglionic terminal, transverses the cellular membranes and, either still combined or free, the nuclear membrane* **(2)**. *In the nucleus, it enhances the transcription of the gene for AChE* **(3)**. *The mRNA enters the cytoplasm and is translated by ribosomes on the rough endoplasmic reticulum* **(4)** *to produce the monomeric* G_1 *form of AChE (5, cross-hatched). A portion of the* G_1 *form is transported to the cellular and dendritic membranes where it is assembled into the* G_4 *tetramer (6, solid line), and a portion of this collects at synaptic sites where it is further aggregated to the collagen-tailed* A_{12} *form (7, dotted). In cholinergic preganglionic fibers of the cat, AChE as the* G_4 *form is present throughout the length of the axon and its terminal, whereas it is present only in traces in adrenergic axons.*

permit its penetration of the effector cell membrane. Although it causes inhibition of spontaneous firing when applied microiontophoretically to cells of the rat brainstem[20], its major documented effect is enhancement of the synthesis of AChE at a stage prior to the assembly of the G_1 monomer into higher polymers. It can be speculated that it does so in a more restricted manner analagous to that of triiodothyronine, a compound of roughly similar size and structure (Fig. 1). The present working hypothesis for the site of action of GlyGln is summarized in Fig. 5.

□ □ □

During the past few decades a host of peptides has been implicated as neurotransmitters or neuromodulators[21]. It is possible that compounds of this class may serve also as neurotrophic factors for the synthesis of specific proteins, as proposed here for GlyGln. To speculate a step further, local deficiencies of such neurotrophic factors may be causally involved in the pathology associated with traumatic and degenerative conditions of the central nervous system.

Acknowledgements

Most grateful thanks are extended to the collaborators in these studies, whose names are listed in the references. Support was provided by the NINCDS, National Institutes of Health, USA, the Centre Nationale de la Recherche Scientifique (CNRS, France), The Barra Foundation, The Burroughs Wellcome Fund, Foundation for Vascular-Hypertension Research (Philadelphia), Hoffmann–La Roche, Inc., Merck Sharp & Dohme Research Laboratories, Ruskin Reseach Foundation, Smith Kline & French Laboratories and Whitehall Laboratories. The manuscript was typed by Cindi Patillo.

References

1 Baxter, J. D. *et al.* (1979) *Recent Prog. Horm. Res.* 35, 97–153
2 Ramsden, D. B. and Hoffenberg, R. (1983) *Clin. Endocrinol. Metab.* 12, 101–115
3 Koelle, G. B. and Ruch, G. A. (1983) *Proc. Natl Acad. Sci. USA* 80, 3106–3110
4 Koelle, G. B., Sanville, U. J., Rickard, K. K. and Williams, J. E. (1984) *Proc. Natl Acad. Sci. USA* 81, 6539–6542
5 Koelle, G. B., Sanville, U. J. and Wall, S. J. (1985) *Proc. Natl Acad. Sci. USA* 82, 5213–5217
6 Koelle, G. B., Sanville, U. J., Thampi, N. G. and Wall, S. J. (1986) *Proc. Natl Acad. Sci. USA* 83, 2751–2754
7 Koelle, G. B., Sanville, U. J. and Thampi, N. G. (1987) *Proc. Natl Acad. Sci. USA* 84, 6944–6947
8 Koelle, G. B., Massoulié, J., Eugène, D., Melone, M. A. B. and Boulla, G. (1987) *Proc. Natl Acad. Sci. USA* 84, 7749–7752
9 Koelle, G. B., Massoulié, J., Eugène, D. and Melone, M. A. B. (1988) *Proc. Natl Acad. Sci. USA* 85, 1686–1690
10 Koelle, G. B., Skau, K. A., Thampi, N. S., Hymel, D. M. and Han, M. S. *Proc. Natl Acad. Sci. USA* (in press)
11 Sawyer, C. H. and Hollinshead, W. H. (1945) *J. Neurophysiol.* 8, 137–153
12 Koelle, W. A. and Koelle, G. B. (1959) *J. Pharmacol. Exp. Ther.* 126, 1–8
13 Koelle, G. B. (1962) *J. Pharm. Pharmacol.* 14, 65–90
14 Davis, R. and Koelle, G. B. (1978) *J. Cell. Biol.* 78, 785–809
15 Davis, R. and Koelle, G. B. (1981) *J. Cell. Biol.* 88, 581–590
16 Kreutzberg, G. W. and Tóth, L. (1974) *Naturwissenschaften* 61, 37
17 Chubb, I. W., Goodman, S. and Smith, A. D. (1976) *Neuroscience* 1, 57–62
18 Chungcharoen, D., Daly, M. de B. and Schweitzer, A. (1952) *J. Physiol. (London)* 118, 528–536
19 Haynes, L. W. and Smith, M. E. (1985) *Biochem. Soc. Trans.* 13, 174–175
20 Parish, D. C., Smyth, D. G., Normanton, J. R. and Wolstencroft, J. H. (1983) *Nature* 306, 267–270
21 Snyder, S. H. (1980) *Science* 209, 976–983