Identification of the probable site of synthesis of butyrylcholinesterase in the superior cervical and ciliary ganglia of the cat

(cholinesterase/propionylcholinesterase/rat/sympathetic nervous system)

Eiji Uchida and George B. Koelle
Department of Pharmacology, Medical School/G3, University of Pennsylvania, Philadelphia, PA 19104

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ABSTRACT The source of butyrylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.8) in the ganglion cells of the cat superior cervical and ciliary ganglia has been elusive, inasmuch as the enzyme is present in high concentrations in the neuropil, where it is confined largely to the dendritic and perikaryonal plasma membranes, but appears to be absent from the perikarya. In the present study, ganglionic butyrylcholinesterase was near-totally inactivated by the injection of tetramonoisopropyl pyrophosphoramide (6.0 μmol/kg of body weight) intravenously. During the ensuing 72 hr, the regenerating enzyme became detectable by the copper thiocynate histochemical method in the somata of essentially all ganglion cells and in the neuropil. Results were similar in pre-ganglionically denervated superior cervical ganglia and in normal ciliary ganglia. These findings suggest (i) that butyrylcholinesterase is indeed synthesized in the ganglion cell perikarya (presumably, the rough endoplasmic reticulum) and transported extremely rapidly to more peripheral cellular sites and (ii) that the synthesis is largely independent of control by any neurotrophic factor provided by the preganglionic axonal terminals. Similar studies were conducted in the rat. In this species, in contrast to the cat, the somata of essentially all ganglion cells of the superior cervical ganglion contain various but at least moderate concentrations of acetylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.7) and propionylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.8). After injection of tetramonoisopropyl pyrophosphoramide, propionylcholinesterase reappeared in the ganglion cell somata before its accumulation in the neuropil, as would be expected.

There is a marked species difference between the cat and rat with regard to the distributions of acetylcholinesterase (AcChoEase; acetylcholine acetylhydrolase, EC 3.1.1.7) and non-specific cholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.8) in the superior cervical ganglion (SCG) and other autonomic ganglia.

In the rat, light microscopic examination by the highly specific copper thiocynate (CuSCn) method has shown that AcChoEase is present in the perikarya of all ganglion cells of the SCG (1); its concentration there is high in a small proportion (<1%; refs. 1 and 2) and moderate or low in the remainder; a high concentration of AcChoEase is also present in the neuropil. The latter is comprised of preganglionic axons and their terminals, ganglion cell dendrites, and Schwann sheath cells; these elements are indistinguishable by light microscopy. However, more recent electron microscopic (EM) examination by the bisthioacetoxycuprate method (3) has clarified the cytological location of AcChoEase in the neuropil (4, 5). The nonspecific cholinesterase (or butyrylcholinesterase; BtChoEase) of the cat SCG appears by light microscopy to be confined to the neuropil and absent from the ganglion cell perikarya (6). It was concluded on this basis and from other studies (7, 8) that it is located in the Schwann sheath cells. However, it was found by EM that most of the BtChoEase is located at the dendritic and perikaryonal membranes of the ganglion cells (4, 5). In view of the apparent absence of BtChoEase from the ganglion cell perikarya cytoplasm, which includes the rough endoplasmic reticulum (RER), this has raised the question of its site of origin. In studies addressed to this point, it was shown that the BtChoEase of the cat SCG is not derived post-transcriptionally from AcChoEase (9) and is not taken up from the plasma (unpublished data).

In this investigation we explored the possibility that BtChoEase is present in the perikaryal cytoplasm of ganglion cells of the cat SCG and ciliary ganglion (CG) in concentrations that are subdetectable by the current CuSCho histochemical procedure (10). This has been done by (i) extending the incubation time from 2 to 8 hr and (ii) examining the sites of regeneration of BtChoEase from 24 to 72 hr after its inactivation by tetramonoisopropyl pyrophosphoramide (iso-OMPA). Results suggest that BtChoEase is synthesized by the RER of the ganglion cells and transported extremely rapidly to their dendritic and perikaryonal membranes.

In contrast to the cat, the rat and most other species show extremely variable but generally moderate concentrations of both AcChoEase and nonspecific cholinesterase [in the rat, propionylcholinesterase (PropChoEase); see ref. 11] in the perikarya of essentially all sympathetic ganglion cells and in the neuropil (12, 13). This suggests that in the rat both enzymes are synthesized in the RER and are then transported to other sites in accordance with current concepts (14). We also examined the localization of PropChoEase in the SCG of the rat by the same procedures as mentioned above for cat ganglia in order to confirm results obtained with the latter species.

METHODS

Cats and rats were anesthetized with sodium pentobarbital (50 mg/kg of body weight) intraperitoneally. The SCG and CG were excised from cats; the SCG was excised from rats. Ganglia were frozen immediately, and fresh frozen sections were cut with a Minitome-Microtome cryostat (International Equipment) at 10 and 20 μm, placed on slides, and stored in the refrigerator overnight; in most cases, the 10-μm sections proved to be more satisfactory. The following day they were stained by the stan-

Abbreviations: AcChoEase, acetylcholinesterase; BtChoEase, butyrylcholinesterase; CG, ciliary ganglion; CuSCho, copper thiocynate; EM, electron microscopy; iso-OMPA, tetramonoisopropyl pyrophosphoramide; PropChoEase, propionylcholinesterase; RER, rough endoplasmic reticulum; SCG, superior cervical ganglion.
standard CuSCho method (10) for AcChoEase and BtChoEase (cat) or PropChoEase (rat). Included in the acetylthiocholine medium for staining AcChoEase was 10-(α-dimethylaminopropionyl)phenothiazine-HCl (designated Astra 1397) at 0.1 mM for the selective inhibition of BtChoEase or PropChoEase; to the medium containing butrylthiocholine for the localization of BtChoEase or PropChoEase was added 1,5-bis-(4-allyldimethylammoniumphenyl)pentan-3-one dibromide (designated B.W. 284) at 3 μM to prevent its slow hydrolysis by AcChoEase. Controls with either substrate contained both inhibitors in the same concentrations. Incubation periods ranged from 1 to 8 hr at 37°C.

In order to determine the initial sites of regeneration of BtChoEase after its essentially complete inactivation, a series of cats was anesthetized with sodium pentobarbital (35 mg/kg) intraperitoneally, and a 1-cm segment was resected from the right cervical sympathetic trunk. Approximately 1 wk later, they were reanesthetized, the saphenous vein was exposed, and they were injected intravenously with iso-OMPA (Ayerst Laboratories, Rouses Point, NY; 6.0 μmol/kg). At intervals of 24, 48, and 72 hr later, they were again anesthetized, and the SCG and CG were taken as above. The regeneration of PropChoEase in the rat was studied similarly by injecting iso-OMPA (20.0 μmol/kg) intraperitoneally 1, 24, 48, and 72 hr prior to removal of the SCG.

RESULTS
When 10-μm sections of cat SCG were stained for AcChoEase by incubation for 1 hr, there was marked staining of the neuropil. The somata of occasional ganglion cells showed heavy staining, and faint, barely discernible staining could be detected in the somata of most of the remainder (Fig. 1A). With increasing incubation periods (2, 4, and 8 hr), staining of the ganglion cell somata became progressively heavier (Fig. 1B–D) and, at the longest period, was distinct in all. The nuclei were faintly stained or unstained. At the same time, the neuropil became grossly overstained and exhibited heavy crystalline deposits. At each of these periods, staining for BtChoEase in the neuropil was likewise marked; however, the ganglion cell somata remained essentially unstained (Fig. 1E–H). Occasional ganglion cell somata appeared at first glance to show staining for BtChoEase, especially at the longer incubation periods. Closer inspection suggested that these were cells that were cut near the surface, so that the staining was actually associated with the perikaryonal and dendritic membranes, as found by EM (4).

In the cat CG, where all ganglion cells give rise to cholinergic postganglionic fibers, the somata of all were markedly stained for AcChoEase at 1 hr (Fig. 2A), and the intensity increased progressively at 2, 4 (Fig. 2B), and 8 hr. Here the neuropil is represented chiefly by the heavily stained dendritic halos that surround the individual ganglion cells. Again, staining for BtChoEase was essentially absent from the somata but intense in the neuropil (Fig. 2C and D).

Control sections of cat SCG and CG, for which both inhibitors were included with either substrate, remained essentially blank after 4- and 8-hr incubation (not shown).

In confirmation of previous findings (12, 13), the patterns of AcChoEase and PropChoEase staining in the SCG of the rat were quite different. After a 1-hr incubation, markedly varying concentrations of both enzymes were detectable in the somata of essentially all ganglion cells and in the neuropil (Fig. 3A and C); staining became more intense after 2 (Fig. 3B and D), 4, and 8 hr (not shown). Again, controls incubated in either substrate with both inhibitors remained blank.

The intravenous injection of iso-OMPA at 6.0 μmol/kg in the cat produced essentially complete, selective inactivation of the BtChoEase of the SCG and CG; 24 hr later, the BtChoEase activity of both ganglia was still less than 1% of controls, and at 72 hr returned to approximately 25% of the control value in the SCG and slightly less in the CG (15). Measurement of the
rate of regeneration after treatment with sarin (isopropylmethylphosphonofluoridate) has shown that the value for BtChoEase in denervated SCG is approximately 60% of the value for normal SCG at all intervals up to 3 wk (9). In the present study, BtChoEase was undetectable in the normal and denervated SCG 24 hr after treatment with iso-OMPA, with 8 hr incubation (Fig. 4 A and D). At 48 hr after iso-OMPA injection, trace amounts of BtChoEase were detectable in the somata of most ganglion cells and in the neuropil of both (Fig. 4 B and E). By 72 hr, there was definite staining for BtChoEase activity in the somata of practically all neurons of the normal and denervated SCG, and considerable staining occurred in the neuropil (Fig. 4 C and F). Staining within the ganglion cell somata and in their surrounding dendritic halos was somewhat heavier in the CG; this is best illustrated in a 20-μm section incubated for 8 hr (Fig. 2E). Controls incubated with Astra 1397 remained unstained.

In the rat, the values for PropChoEase in the SCG after the intraperitoneal injection of 20 μmol of iso-OMPA per kg at 2, 24, 48, and 72 hr were 1, 18, 49, and 53% of control values, respectively (16). Here, no staining was found 1 hr after iso-OMPA injection (Fig. 5A). Slight staining was detectable in most ganglion cell somata at 24 hr (Fig. 5B). At 48 hr (Fig. 5C) and 72 hr (Fig. 5D) after iso-OMPA injection, staining increased progressively in the somata and appeared in the neuropil; at the latter period, there was an extremely high proportion of heavily stained ganglion cells. The relative intensity of staining of ganglion cell somata to that of the neuropil at both intervals was considerably greater than in the controls (Fig. 3 C and D).

**DISCUSSION**

The apparent absence of BtChoEase from the somata of the ganglion cells of the cat SCG has been a matter of puzzlement, particularly after the EM demonstration that its presence in the neuropil is restricted almost exclusively to the ganglion cell dendritic and perikaryonal membranes (4). AcChoEase is located at the same sites as well as at the plasma membranes of the preganglionic axons and their terminals (4). The localization of AcChoEase at the ganglion cell somata (1) has been assumed to account for its source at more peripheral sites in the same cells.

In the present study, the foregoing findings were confirmed and extended. Then it was shown that after near-total inactivation of the BtChoEase of the SCG and CG by iso-OMPA, the enzyme became detectable in the ganglion cell somata practically simultaneously with its reappearance in low concentrations in the neuropil. The sum of these findings suggests that BtChoEase is indeed synthesized in the ganglion cell somata (presumably, the RER) and transported extremely rapidly to the perikaryonal and dendritic membranes. Under normal conditions, it is likely that the velocity of this sequence is too rapid to allow the accumulation of histochemically detectable concentrations of BtChoEase in the somata.

The effects of preganglionic denervation of the cat SCG deserve mention. Within 72 hr after this procedure, the AcChoEase disappears completely from the neuropil but not from...
the ganglion cell somata, and BtChoEase is lost partially from the former site; these changes persist for several weeks (10, 17). Recent findings suggest that these effects are due to the loss of a neurotrophic factor provided normally by the preganglionic terminals (18). In the present study, there appeared to be no difference between the appearance of BtChoEase in the somata of normal or preganglionically denervated cat SCG neurons after iso-OMPA injection. This indicates that the perikaryon synthesis of BtChoEase, like AcChoEase, is at least partially independent of neurotrophic control.

In the rat SCG, the situation is much less complicated. Here, PropChoEase, like AcChoEase, is present in varying but significant concentrations in the somata of essentially all ganglion cells. After its inactivation by iso-OMPA, PropChoEase reappeared first in the ganglion cell somata and subsequently in the neuropil, as would be expected.

The bithioacetoxy aurate method, in contrast to the CuSCho procedure, permits high resolution of the localization of AcChoEase and BtChoEase by EM but is relatively nonspecific. Accordingly, the positive identification of either enzyme at any site is dependent upon the absence of significant concentrations of other esterases that are resistant to inhibition by physostigmine (3). Current results indicate that such enzymes are present in the RER of cat sympathetic ganglion cells, in contrast to their relative absence at the perikaryonal and dendritic membranes (4); hence, only at the latter sites can AcChoEase and BtChoEase be identified with certainty.

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