Acetylcholine activity in an identified 5-hydroxytryptamine containing neuron

Each giant metacerebral neuron (or giant serotonin cell-GSC) of *Helix pomatia* contains about 1 ng of 5-hydroxytryptamine (5-HT) (Cottrell & Osborne, 1970; Osborne & Neuhoff, 1973); homologous neurons of related species also contain 5-HT (Osborne & Cottrell, 1971; Weinreich, McCaman & others, 1973).

Electrophysiological studies have shown that each GSC makes synaptic connexions with other identified neurons in the buccal ganglia of *H. pomatia* and pharmacological studies suggest that 5-HT is the transmitter substance used at these synapses (Cottrell, 1970; Cottrell & Macon, 1974 and cf. Paupardin-Tritsch & Gerschenfeld, 1973).

A recent unexplained finding is that the GSC perikarya also contain choline acetyltransferase activity (Hanley, Cottrell & others, 1974). The level of choline acetyltransferase activity in the GSCs was intermediate between those neuron perikarya containing high activity and those, the majority, with no detectable activity.

We wish to report that acetylcholine activity has now also been detected in extracts of the perikarya of the GSCs prepared in eserinized solutions (10^{-4} M) by bioassay using clam (*Mya arenaria*) heart strips (Cottrell, Powell & Stanton, 1970). Extracts of 50 μl of filtered sea water containing one or two GSCs were frozen and thawed five or more times using liquid nitrogen to release any bound acetylcholine. The acetylcholine activity was rapidly lost in non-eserinized solutions and it was destroyed in eserinized solutions by heating for 5 min at an alkaline pH. The inhibitory effect on the heart was antagonized in a quantitatively similar manner to acetylcholine by the known antagonist Mytolon+(Stirling Winthrop). The average level of acetylcholine based on 7 quantitative experiments was 109 $\pm$ 25 pg (s.d.) per GSC, i.e. roughly one tenth the level of 5-HT detected in these neurons.

The precise location of the material remains to be determined. It is unlikely that it is associated with incoming synaptic terminals because all the synaptic contacts are axo-axonic and positioned some distance from the perikarya (Pentreath, Osborne & Cottrell, 1973)).

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† Benzoquinonium chloride.

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