J Physiology (Paris) (1998) 92, 53–57

Arcachon and cholinergic transmission

Victor P. Whittaker*

Max-Planck-Institut für biophysikalische Chemie, D-37070 Göttingen, Germany

Abstract — The cholinergic nature of transmission at the electromotor synapse of *Torpedo marmorata* was established at Arcachon in 1939 by Feldberg, Fessard and Nachmansohn (J. Physiol. (Lond.) 97 (1939/1940) 3P–4P) soon after transmission at the neuromuscular junction had been shown to be cholinergic. In 1964, after a quarter of a century of neglect, workers in Cambridge, then in Paris, Göttingen and elsewhere, began to use this system, 500–1000 times richer in cholinergic synapses than muscle, for intensive studies of cholinergic transmission at the cellular and molecular level. (©Elsevier, Paris)

Resumé — Arcachon et la transmission cholinergique. La nature cholinergique de la transmission à la synapse électromotrice de la torpille a été établie à Arcachon en 1939 par Feldberg, Fessard et Nachmansohn (J. Physiol. (Lond.) 97 (1939/1940) 3P–4P), peu après la découverte du caractère cholinergique de la transmission à la jonction neuromusculaire. Après une période assez longue de désuétude, les chercheurs de Cambridge, Paris, Göttingen et d'ailleurs ont commencé, vers 1964, à utiliser ce système, 500–1000 fois plus riche en terminaisons cholinergiques que le muscle, pour étudier intensivement la transmission cholinergique au niveau cellulaire et moléculaire. (©Elsevier, Paris)

***Torpedo marmorata* / electric organ / electromotor synapse / cholinergic transmission**

1. Introduction

The electric ray, *Torpedo marmorata*, is fairly common in the Bay of Biscay (Baie de Gascoigne): although a slow breeder (6–12 young every 2 years), it is not fished commercially. Its chief interest for the neurobiologist is that it possesses paired electric organs on each side of the midline which are capable of giving shocks (up to 40 V in air), strong enough to stun prey and deter predators. These organs are under the control of the electromotor nerves which originate in the electric lobes, paired nuclei on the brain stem placed caudally to the cerebellum. The electromotor neurons are in turn under the control of the oval nucleus in the brain-stem. This integrates sensations of hunger, detection of prey and nociceptive stimuli and decides whether to release a train of electric shocks (short trains at a frequency of 100–300 Hz). The electric organ consists of stacks of flattened cells known as electrocytes, electroplaques or electroplax; these are densely innervated on their ventral surfaces by fine branches of the electromotor axons.

Torpedo spp. are not the only fish to have electric organs; members of at least seven other diverse families have acquired them by a process of convergent evolution (for a recent review see [21]). However, in terms of availability, amount of tissue per specimen and density of synaptic material, the Torpedinidae, especially *T. marmorata* (Eastern Atlantic), *T. ocellata* (Mediterranian) and *T. californica* (Pacific), are the family of choice.

Electric organs have excited the interest of biologists since earliest times: the puzzling nature of their discharge was only resolved with the discovery of electricity and indeed led to the invention of the battery or Voltaic pile which A. Volta (for attributions in this form, see Whittaker [20] for original references) himself described, in a memoire to the Royal Society of London published in 1800, as an artifical electric organ. J. Bernstein correctly postulated in 1912 that the discharge was caused by a transient increase in the permeability of the electroplaque membrane to positively charged ions.

It has been known since the 1870s, mainly from the work of A. Babuchin in 1876, that the electrocytes are derived embryonically from myoblasts; it is therefore not surprising that the electromotor synapse is cholinergic. Grundfest [5] in a definitive review, reinterpreted Bernstein's theory of the discharge in the light of contemporary knowledge of the excitation of muscle; he described it as the summation in series and in parallel of normal excitatory postsynaptic potentials (EPSPs) evoked by the release of transmitter from the presynaptic nerve terminals. Unlike some other electric organs, the electrocytes of Torpedinidae are electrically inexcitable; thus the discharge in this type of electric organ is

**Address for correspondence*: 197 Huntingdon Road, Cambridge CB3 0DL, UK

pure summed EPSP unmodified by a conducted response corresponding to a muscle action potential.

Apart from a few autonomic vascular endings, the innervation of the *Torpedo* electric organ is purely cholinergic with a synaptic content 500–1000 times that of muscle; indeed the organ resembles a huge mass of hypertrophied motor endplates. With 400–500 g of tissue per fish to work with, the electric organ is an ideal resource for studies of the cellular and molecular biology of all aspects of cholinergic transmission: the synthesis and storage of transmitter, its release and postsynaptic action, its hydrolysis to inactive products (choline and acetate) and their eventual reutilization. The electric lobes, though available in smaller quantities (ca. 400 mg/specimen), contain large (~40 μm diameter) cholinergic cell bodies studded with putative glutamergic nerve terminals; they contain the complete genome for cholinergic function and the mechanisms for the formation and transport of synaptic vesicles. In only one important respect does the electromotor terminal differ from terminals in muscle (including *Torpedo* muscle): its synaptic vesicles are larger (~90 nm in diameter versus ~50 nm in muscle). This facilitates a study of their function which appears to be similar in all respects to that of their smaller congeners.

2. Discovery of the cholinergic nature of transmission at the electromotor synapse

The discovery that transmission at the electromotor synapse is cholinergic was made during 3 weeks of research in June 1939 as the coming World War cast its shadow over Europe. Three neurobiologists of very different backgrounds came together at the Station Biologique d'Arcachon where *Torpedo* from the Bassin was readily available. They were A. Fessard, an electrophysiologist from the Collège de France, Paris, D. Nachmansohn, a biochemist and German Jewish emigrant working at the Sorbonne, Paris, and W. Feldberg, a pharmacologist and also a German Jewish emigrant working with H.H. Dale in London.

Fessard had worked extensively with electric tissue (for literature citations see Feldberg and Fessard [4]). He showed that excitation could only be brought about via the nerve – denervated tissue was unexcitable mechanically and electrically – and that nerve action could be blocked by drugs (curare, eserine) that block the cholinergic transmission in muscle discovered by H.H. Dale, W. Feldberg and M. Vogt in 1936. He surmised that the discharge must be triggered by the release of a depolarizing substance from the electromotor nerve terminals, probably acetylcholine, as in muscle.

Nachmansohn, a medical graduate of Berlin University, had received his early research training with O. Meyerhof, who had done classical work on the energy-yielding metabolic reactions sustaining muscular contractions. Nachmansohn thought that biochemical methods should be applied to nerve activity and argued that if acetylcholine were indeed a transmitter at the neuromuscular junction, the released transmitter must be destroyed by cholinesterase within the refractory period of muscle. With his student-assistant Annette Marnay he showed that the cholinesterase activity of the innervated portion of the frog sartorius muscle was several thousand times greater than that of the non-innervated portion and great enough to destroy released acetylcholine within the muscle's refractory period. Being aware that the electrocytes of electric tissue resembled motor endplates, he asked Marnay to test for the presence of cholinesterase in electric tissue. She found very high levels of activity in this tissue, exceeding that of muscle several hundred-fold.

Fessard heard about these results and invited Nachmansohn to work with him at Arcachon on chemical transmission in the electric organ. However, if the techniques the Dale group had used so successfully with muscle were to be applied to the electric organ, a third collaborator who could assay acetylcholine in tissue and perfusates and apply it to the organ by close arterial injection would be needed.

Accounts differ [20] on how Feldberg came to be invited to join Fessard and Nachmansohn but no-one better qualified than he could have been chosen, in view of his participation in the work on muscle.

The results showed unequivocally that transmission at the electromotor synapse is cholinergic. Publication was delayed by the outbreak of the Second World War in September 1939 and Nachmansohn's hasty departure for America, but the results were eventually written up by Feldberg and were published, together with some additional work Feldberg did in London on extracts of electric organ he brought back with him, in a now classical paper in the Journal of Physiology [4].

Three lines of evidence justify the conclusion that transmission at the electromotor synapse is cholinergic. Firstly, extracts of electric organ under condtions which prevent the breakdown of acetylcholine contain a substance which precisely matches the action of acetylcholine in a variety of assay systems: the frog rectus abdominis muscle; the dorsal muscle of the leech; slowing of the frog's heart; a fall in the cat's blood pressure; adrenaline release from the adrenal medulla. No other known substance would

match acetylcholine in all of them. The substance behaved like acetylcholine in its lability in alkaline solutions and its sensitivity to cholinesterase. The acetylcholine equivalence of the substance (400 nmol g of tissue^{-1}) is within the range found by later authors. In the second line of evidence it is shown that what we can now assume to be acetylcholine is released into perfusates of the organ on stimulation and in the third, close arterial injection of authentic acetylcholine excited discharges similar to those evoked by nerve stimulation.

Some years later Woodin (cited by Whittaker [19]) confirmed the identity of the acetylcholine-like substance in electric organ as acetylcholine by paper chromatography and more recently, it has been unequivocally identified by gas chromatography-mass spectrometry [18].

Nachmansohn continued to work on electric organs in the US, but switched to the electric eel, *Gymnotus electricus*, a denizen of the Amazon. His views on the role of acetylcholine in the nervous system began to deviate sharply from those of Dale and most other neurobiologists (for a critique and references, see Whittaker [20]); nevertheless, his group made many important contributions to cholinergic neurobiology. The comparative electrophysiology of electric organs attracted sporadic attention during the 1950s and with the coming of electron microscopy, the fine structure of the electromotor synapse was studied in several Torpedinidae: *Narcine* [17]; *T. marmorata* [9, 13]. Nevertheless, the potentialities of the electromotor system for the investigation of the cellular and molecular biology of cholinergic transmission remained largely neglected for 25 years.

3. The electromotor innervation of *Torpedo* as a model cholinergic system

3.1. The electromotor synapse: cytoplasmic and vesicular pools of acetylcholine

My own involvement with the *Torpedo* electric organ started in 1963 soon after my discovery of the synaptosome and the successful isolation from synaptosomes of pure preparations of mammalian cortical synaptic vesicles, a proportion of which stored acetylcholine in amounts consistent with their being the morphological basis of quantal transmitter release [24, 26]. In 1963 a colleague, R.D. Keynes, suggested that *Torpedo* electric organ might be a better source of cholinergic vesicles than mammalian brain. The tissue proved difficult to homogenize, but with M.N. Sheridan and M. Israël [14, 27] synaptosomes in low yield and reasonably homogeneous preparations of synaptic vesicles were obtained. Purer preparations of the latter were subsequently obtained by M. Israël on his return to France. Meanwhile, the large-scale isolation of pure synaptic vesicles was made possible [25] by three technical innovations: comminution of the tissue by crushing after rendering it brittle by freezing it at low temperatures; extraction of the crushed tissue with solutions iso-osmotic to *Torpedo* fluids; and high-resolution separation of the resultant cytoplasmic extracts on continuous density gradients in a large-capacity zonal rotor. This enabled the composition, biophysics and recycling of synaptic vesicles to be intensively studied [20]. Briefly, it was established by the Göttingen group that acetylcholine exists in two pools: the cytoplasmic, where it is synthesized, and the vesicular, in which it is stored and from which it is released. Small differences in density and composition enabled three pools of vesicles to be separated: largely empty vesicles newly arrived from the cell body by fast axonal transport [6], a reserve pool and a stimulus-induced recycling pool [29] which preferentially takes up acetylcholine newly synthesized in the cytoplasm. By using choline analogues it was possible to label the cytoplasmic and the two transmitter-containing vesicular pools differentially and to show that transmitter released by stimulation arises exclusively from the recycling pool [7].

The vesicular uptake of acetylcholine is driven by a proton gradient generated by a vacuolar-type ATPase [28] and is facilitated by a vesicular acetylcholine transporter (vAChT), the sequence of which is encoded within the first intron of the gene locus for choline acetyltransferase [3].

3.2. Synaptosomes and presynaptic plasma membranes

Improved methods of preparing synaptosomes were developed both by our group and that of Israël; when well sealed these were able to develop membrane potentials comparable to those in intact neurons [10, 11]. The specificity and properties of the choline uptake system were extensively studied [2, 23]. The plasma membranes are rich in a family of minor gangliosides containing a sialylated *N*-acetylgalactosamine residue; these are specific for mammalian cholinergic neurons and have permitted the immunoisolation of cholinergic synaptosomes from a mixed population (for review see [22]).

3.3. Electromotor cell bodies and axons

The cell bodies of electromotor neurons are ~40 μm in diameter and thus among the largest ver-

tebrate motor neurons known. They are readily isolated and though shorn of their dendrites are well sealed. Their choline acetyltransferase activity is twice that of cholinergic cell bodies in *Aplysia* and about 40 times greater than mammalian ganglion cells, but only one quarter that of electromotor nerve terminals.

The perikarya of these cells are rich in Golgi membranes and synaptic vesicles and contain readily translatable mRNA which code inter alia for several synaptic vesicle proteins [12]. Choline acetyltransferase is transported down the axon at the slow rate [1]; synaptic vesicles are transported at the fast rate and take up acetylcholine only when they reach the terminal [6].

The nerve terminals in the lobe are those of afferents from the command or oval nucleus. They are probably glutamergic and have been isolated in low yield by conventional methods.

3.4. Cotransmission in the electromotor synapse

Many cholinergic neurons are now known to utilize various neuropeptides as co-transmitters. The electromotor neurons are no exception; as in mammalian brain and autonomic ganglia, VIP is the co-transmitter. It is transported to the terminal in dense-cored vesicles (for references see Whittaker [20]). Another vesicular constituent which may act as a cotransmitter or regulator is ATP, present in vesicles in a 1:5 ratio to acetylcholine.

3.5. The nicotinic acetylcholine receptor (nAChR)

In one of the major advances of modern neurobiology the acetylcholine receptor in the muscle and electrocyte postsynaptic membrane is now known to be a ligand-gated pentameric ion channel composed of four polypeptide subunits, α, β, γ, δ of known sequences in which the α unit is represented twice. Early work was done on *Electrophorus* (*Gymnotus*) electric organ but when it was realised (from about 1975) that *Torpedo* membranes are much richer in receptor than *Electrophorus*, there was a shift to *Torpedo* spp. (*T. californica* and *T. marmorata*) by the many groups working in this field. The complete receptor is a rivet-like structure which undergoes a tilting conformational change as it opens and closes [16].

3.6. Acetylcholinesterase

The type of cholinesterase present in electrocytes hydrolyses acetylcholine faster than other choline esters and is thus known as an acetylcholinesterase. It has been fully sequenced and its tertiary structure and mode of catalysis are known. The choline moiety of acetylcholine is firmly held in a trough lined with 14 aromatic residues whose π electrons interact with the positive charge on the quaternary nitrogen of acetylcholine. Hydrolysis is effected by a charge relay system; the catalytic triad has been identified as Glu-327, His-440 and Ser-200. These widely separated residues are brought together by folding [15].

Massoulié and coworkers (for review see Massoulié and Toutant [8]) have shown that acetylcholinesterase can exist in several forms in electric organ and other tissues. The catalytic subunit (G_1) is a highly glycosylated soluble globular protein of molecular mass 70–85 kDa. In tissues a tetramer (G_4) of this unit is normally conjugated through S-S bonds to a collagen tail giving an asymmetric form (A_4). Further aggregation via interaction of the tails gives larger forms culminating in A_{12}, an aggregate of three A_4 units. Such forms are extractable by solutions of high ionic strength. In another conjugate, dimeric catalytic units are linked through ethanolamine, glycan and glucosamine to phosphatidylinositol (PI); this form is inserted via the lipid end of its tail into lipoprotein membranes. Such tails are found in several unrelated membrane-bound molecules with the common property of being solubilized by PI-specific phospholipase C (PIPLC).

4. The development of the electromotor system

This has been extensively studied by J. Melllinger in Rheims and by the Göttingen group (for references see Whittaker [20]). Nineteenth-century observations have been confirmed and extended by electronmicroscopic, electrophysiological and biochemical techniques. Briefly, four stages in development have been recognized: I, myogenic; II, electrocytogenic; III, synaptogenic divided into IIIa, penetration of stacks of electrocytes by ingrowing neurites, and IIIb, functional synaptogenesis. During phases II and IIIa, extensive apoptosis of electromotor neurons takes place as neurites are matched with their targets. Two trophic factors have been identified: a heat-stable neuronotrophic factor essential for neuronal survival and a heat-sensitive cholinotrophic factor that stimulates the expression of the cholinergic phenotype. The molecular structure of these factors has not been identified.

5. Conclusion

Although a vast amount of important work has been done with the electromotor system of *Torpedo*

since its cholinergic character was first established by Feldberg, Fessard and Nachmansohn, its potentiality is far from exhausted. While fish may not be so convenient to work with as small rodents, any difficulties in transporting and keeping them can be overcome by careful organization.

'Cholinologists' have reason to be grateful to the Station Biologique d'Arcachon for their exceptional willingness to provide *Torpedo* alive or as frozen tissues to so many laboratories and for the local organization and facilities which have made this possible.

References

[1] Davies L.P., ATP in cholinergic nerves: evidence for the axonal transport of a stable pool, Exp. Brain Res. 33 (1978) 149–157.

[2] Ducis I., Whittaker V.P., High-affinity sodium-gradient-dependent transport of choline into vesiculated presynaptic plasma membrane fragments from the electric organ of *Torpedo marmorata* and reconstitution of the solubilized transporter into liposomes, Biochim. Biophys. Acta 815 (1985) 109–127.

[3] Erickson J.D., Varoqui H., Schäfer M.K.H., Modi W., Diebler M.F., Weihe E., Rand J., Eiden L.E., Bonner T., Usdin T.B., Functional identification of a vesicular acetylcholine transporter and its expression from a 'cholinergic' gene locus, J. Biol. Chem. 269 (1994) 21929–21932.

[4] Feldberg W., Fessard A., The cholinergic nature of the nerves to the electric organ of the *Torpedo (Torpedo marmorata)*, J. Physiol. 101 (1942) 200–216.

[5] Grundfest H., The mechanism of discharge of the electric organ in relation to general and comparative electrophysiology, Prog. Biophys. Chem. 7 (1957) 1–85.

[6] Kiene M.L., Stadler H., Synaptic vesicles in electromotoneurones. I. Axonal transport, site of transmitter uptake and processing of a core proteoglycan during maturation, EMBO J. 6 (1987) 2209–2215.

[7] Luqmani Y.A., Sudlow G., Whittaker V.P., Homocholine and acetylhomocholine: false transmitters in the cholinergic electromotor system of *Torpedo,* Neuroscience 5 (1980) 153–160.

[8] Massoulié J., Toutant J.P., Vertebrate cholinesterases: structure and types of interaction, in: Whittaker V.P. (Ed.), The Cholinergic Synapse, Springer Verlag, Berlin,1988, pp. 167–224.

[9] Mellinger J., Belbenoît P., Ravaille M., Szabo T., Electric organ development in *Torpedo marmorata*, Chondrichthyes, Dev. Biol. 67 (1978) 167–188.

[10] Meunier F.M., Relationship between presynaptic membrane potential and acetylcholine release in synaptosomes from *Torpedo* electric organ, J. Physiol. 354 (1984) 121–137.

[11] Richardson P.J., Whittaker V.P., The Na^+ and K^+ content of isolated *Torpedo* synaptosomes and its effect on choline uptake, J. Neurochem. 36 (1981) 1536–1542.

[12] Schmid D., Stadler H., Whittaker V.P., The isolation, from electromotor neuron perikarya, of messenger RNAs coding for synaptic proteins, Eur. J. Biochem. 122 (1982) 633–639.

[13] Sheridan M.N., The fine structure of the electric organ of *Torpedo marmorata*, J. Cell Biol. 24 (1965) 129–141.

[14] Sheridan M.N., Whittaker V.P., Isolated synaptic vesicles: morphology and acetylcholine content, J. Physiol. 175 (1964) 25P–26P.

[15] Sussman J.L., Harel M., Frolow F., Oefner C., Goldman A., Toker L., Silman I., Atomic structure of acetylcholinesterase from *Torpedo californica*: a prototypic acetylcholine-binding protein, Science 253 (1991) 872–879.

[16] Unwin P.N.T., Toyoshima C., Kubalek E., Arrangement of the acetylcholine receptor subunits in the resting and desensitized states, determined by cryoelectron microscopy of crystallized *Torpedo* postsynaptic membranes, J. Cell Biol. 107 (1988) 1123–1138.

[17] Wachtel A., Mathewson R., Grundfest H., Electron microscopic and histochemical comparison of the two types of electroplaques of *Narcine brasiliensis*, J. Biophys. Biochem. Cytol. 11 (1961) 663.

[18] Weiler M., Roed I.S., Whittaker V.P., The kinetics of acetylcholine turnover in a resting cholinergic nerve terminal and the magnitude of the cytoplasmic compartment, J. Neurochem. 38, (1982) 1187–1191.

[19] Whittaker V.P., Identification of acetylcholine and related esters of biological origin, in: Koelle G.B. (Ed.), Cholinesterases and Anticholinesterase Agents, Springer Verlag, Berlin, 1963, pp. 1–39.

[20] Whittaker V.P., The Cholinergic Neuron and its Target: The Electromotor Innervation of the Electric Ray *Torpedo* as a Model, Birkhäuser Verlag, Boston MA, 1992.

[21] Whittaker V.P., Electric organs and their innervation: a model system for the study of cholinergic function, in: Walz D., Berg H., Milazzo G. (Eds.), Bioelectrochemistry of Cells and Tissues, vol 2, Birkhäuser Verlag, Basel, 1995, pp. 1–33.

[22] Whittaker V.P., Kelić S., Cholinergic-specific glycoconjugates, Neurochem. Res. 20 (1966) 1377–1387.

[23] Whittaker V.P., Luqmani Y.A., False transmitters in the cholinergic system: implications for the vesicle theory of transmitter storage and release, Gen. Pharmacol. 11 (1980) 7–14.

[24] Whittaker V.P., Sheridan M.N., The morphology and acetylcholine content of isolated cerebral cortical synaptic vesicles, J. Neurochem. 12 (1965) 363–372.

[25] Whittaker V.P., Essman W.B., Dowe G.H.C., The isolation of pure cholinergic synaptic vesicles from the electric organs of elasmobranch fish of the family Torpedinidae, Biochem. J. 128 (1972) 883–846.

[26] Whittaker V.P., Michaelson I.A., Kirkland R.J.A., The separation of synaptic vesicles from nerve ending particles ('synaptosomes'), Biochem. J. 90 (1964) 293–305.

[27] Whittaker V.P., Sheridan M.N., Israël M., The subcellular fractionation of the electric organ of *Torpedo*, Z. Zellforsch. 74 (1966) 291–307.

[28] Yamagata S.K., Parsons S.M., Purification and subunit composition of a cholinergic synaptic vesicle glycoprotein, phosphointermediate-forming ATPase, J. Neurochem. 53 (1989) 1345–1353.

[29] Zimmermann H., Denston C.R., Separation of synaptic vesicles of different functional states from the cholinergic synapses of the *Torpedo* electric organ, Neuroscience 2 (1977) 715–730.