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High-resolution autoradiographic study of α-Bungarotoxin receptors in the developing chick ciliary ganglion (**)

RIASSUNTO. — Gangli ciliari di embrione di pollo sono stati incubati con [^{125}I] α-Bungarotoxina, una neurotossina che si lega ai recettori nicotinici per l'acetilcolina. La distribuzione istologica di tali recettori è stata poi studiata mediante autoradiografia al microscopio elettronico. Mentre una parte dei recettori osservati è situata in corrispondenza dei contatti sinaptici ed è verosimilmente da correlare alla neurotrasmissione gangliare, altri recettori sono caratteristicamente localizzati in aree neuronali extrasinaptiche. Ulteriori indagini saranno necessarie per chiarire il ruolo funzionale dei recettori extrasinaptici.

It is well known that α-Bungarotoxin (α-BuTX) a neurotoxin obtained from the venom of *Bungarus multicinctus*, binds with high affinity to nicotinic acetylcholine receptors (AChRs) blocking irreversibly the synaptic transmission in skeletal muscle (Chang and Lee, 1963) and electroplax (Changeux *et al.*, 1970). Recently AChRs have been found also in autonomic ganglia (Greene *et al.*, 1973; Fumagalli *et al.*, 1976), their functional role being still to be elucidated. In a previous paper we reported the presence of α-BuTX binding sites in chick embryo ciliary ganglia (Gangitano *et al.*, 1978). Here we describe the electron-microscope autoradiographic distribution of these binding sites in the 12 days-old chick embryo ciliary ganglion.

Desheathed chick embryo ciliary ganglia were incubated for 2 h with 60 nM [^{125}I] α-BuTX (specific activity: 162 Ci/mmol) in the presence or in the absence of 0.5 mM d-tubocurarine (dTC). Unbound toxin was removed by extensive washing.

After binding assay, the pre-treated ciliary ganglia were fixed in glutaraldehyde, post-fixed in osmium, dehydrated in ethanol and embedded in Epon. Ultrathin

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sections from embedded ganglia were coated with Ilford L4 emulsion by the loop technique, exposed at 4 °C for two months and developed with the Gold–Latensification-Phenidon method, according to Bouteille (1976). The resolving power of our autoradiographic method was 900 Å, according to Salpeter *et al.* (1977). The background density of silver grains was less than 1 grain/100 μm^2.

At this developmental stage — i.e. 12 days-old chick embryo —, the two cell populations typical of the avian ciliary ganglion — i.e. the ciliary and the choroid neurons (Marwitt *et al.*, 1971) — are not yet sharply differentiated. That's why our autoradiographic evaluation deals with both cell types.

The silver grains are discontinuously localized along the neuronal plasma membrane and mainly arranged in patches of higher concentration (Fig. 1). In particular, they are observed at the level of synaptic contacts, superimposed on the pre- and the post-synaptic membrane (Fig. 2). Areas are observed in which the radioactivity seems not to be exclusively localized over the neuronal plasma membrane but also over the interdigitating neuronal and satellite processes occurring near the neuronal surface (Fig. 3). A small number of silver grains is present also over the peripheral areas of the neuronal cytoplasm without any constant relationship with definite cytoplasmic structures (Fig. 1, 2). These silver grains could be related to intracytoplasmic binding sites (Fambrough and Devreotes, 1978) although the possibility that they are internalized by a pinocytotic mechanism cannot be ruled out. The marked reduction in number of silver grains following incubation with labelled toxin in the presence of 0.5 mM dTC would confirm that silver grains are really related to nicotinic receptors. (Fig. 4).

Binding of α-BuTX to nicotinic receptors has been recently observed in the adult and embryonic chick ciliary ganglion (Fumagalli *et al.*, 1978; Gangitano *et al.*, 1978; Chiappinelli and Giacobini, 1978).

In the present experiments we have studied the autoradiographic localization of AChRs in the embryonic ciliary ganglion. In this context we have focused our attention on the 12 days-old chick embryo, a critical developmental stage characterized by a remarkable increase in number of AChRs (Gangitano *et al.*, 1978). AChRs are often detected at the level of the synaptic contacts. Interestingly, in this very period of embryonic life, the specific acetylcholinesterase activity which is regarded as a marker of synaptic maturation, remarkably increases (Chiappinelli *et al.*, 1976). Furthermore, in the adult chick ciliary ganglion — differently from other autonomic ganglia — a blockade of transmission by α-BuTX has been recently found (Chiappinelli and Zigmond, 1978). These findings taken together strongly suggest that these toxin binding sites are related to the ganglionic transmission. Interestingly, other AChRs are present in extrasynaptic areas, similarly to what observed in muscle (Fambrough, 1979) and electroplax (Bourgeois *et al.*, 1972). On the other hand, extrasynaptic AChRs sensitive to α-BuTX have been found also in other autonomic ganglia (Dun and Karczmar, 1980).

It may be suggested that in our case the extrasynaptic receptors undergo a lateral motion on the neuronal surface towards the synaptic areas, there possibly taking part in ganglionic transmission. There are, however, different opinions about

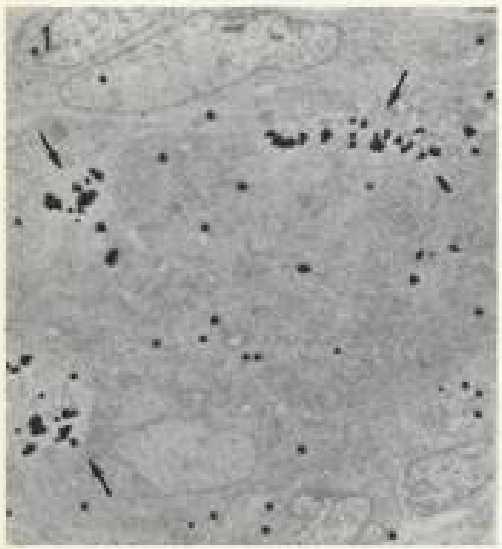

Fig. 1. — Electron microscope autoradiography of a 12 day-old chick embryo ciliary ganglion labelled with 60 nM [^{125}I] α-BuTX. A neuron shows peripherally localized patches of radioactivity (arrows). Scattered silver grains are present in the cytoplasm. × 4500

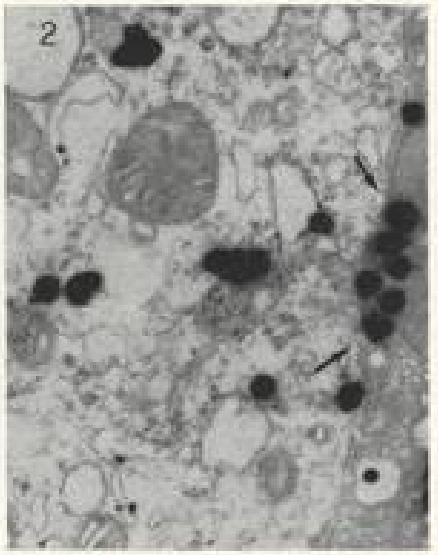

Fig. 2. — A synaptic contact is heavily labelled with radioactivity. Some silver grains are present in the peripheral cytoplasm.
× 27000

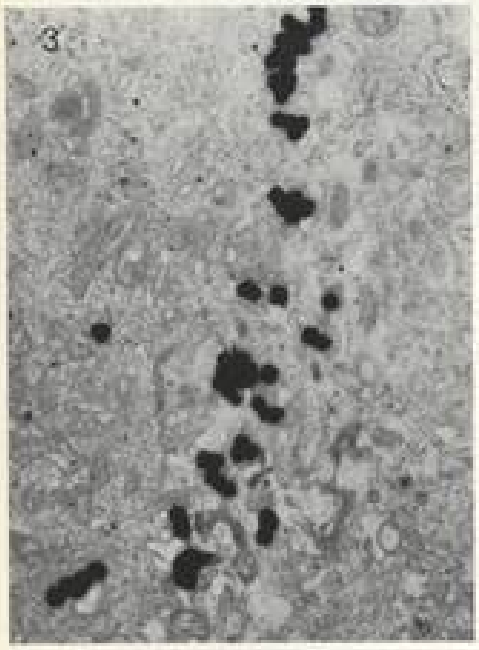

Fig. 3. — Several radioactive patches are observed along the neuronal plasma membrane and over the interdigitating neuronal and satellite processes. × 13900

Fig. 4. — [^{125}I] α-BuTX bound to 12 day-old chick embryo ciliary ganglion in the presence of 0.5 mM dTC (non specifically bound toxin). Only scattered silver grains are present in the neuronal pericarion.
× 8200

the possibility of an intramembranous movement of AChRs, at least as regards the dynamic behaviour of AChRs on the membranes of cultured myotubes (Axelrod, 1976). Besides the possibility that such receptors are involved in other biological events, cannot be ruled out. In conclusion, the functional role of the extrasynaptic AChRs needs further investigation.

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