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DISTRIBUTION OF [^{125}I]-α-BUNGAROTOXIN BINDING PROTEINS IN FRACTIONS FROM BULL SPERMATOZOA

B. V. Rama Sastry, Michael R. Bishop and Sen T. Kau

Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232, U.S.A.

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Propulsion of spermatozoa depends on the precise coordination of a propagated flagellar wave (1,2). Nelson (3) describes the relative arrangement of contractile fibers in spermatozoa, which is similar to that of cilia, i.e. 9 + 9 + 2. The outer large (γ) fibers are the contractile proteins, while the central pair (β) and smaller (α) fibers (droplets adjacent to γ fibers) are probably related to conduction and coordination of the contractile system (3,4). It is possible that the function of a cholinergic system consisting of choline acetyltransferase (ChA), acetylcholine (ACh), acetylcholinesterase (AChE) and cholinergic receptor (AChR) is coupled to the excitation and relaxation phases of the contractile apparatus in spermatozoa.

Spermatozoa from bull have been shown to contain ChA (5,6), ACh (5,6) and AChE (7,8). Spermatozoa from the sea urchin, Arbacia punctulata, have been shown to contain AChR of the nicotinic type, where nicotine (> 10^{-7} M) and α-bungarotoxin (5 x 10^{-8} M) interact and decrease sperm motility (9,10). The presence of one or more components of the cholinergic system has been demonstrated in spermatozoa of several mammalian and marine invertebrate species (2).

The subcellular distribution of ChA and AChE in bull sperm fractions of heads, midpieces and tails is similar (6,7). Specific activities of ChA and AChE fall in the following order: tails > midpieces > heads. In view of the small quantities of ACh in bull spermatozoa and the lack of storage sites for ACh in spermatozoa, the distribution of ACh in sperm fractions could not be determined (7). However, if the spermatozoal cholinergic system is important for sperm motility, the distribution of AChR of the nicotinic type should follow the same distribution of ChA and AChE in sperm fractions. α-Bungarotoxin, a component of the venom of Bungaris multicinctus, binds to AChR of the nicotinic type and produces a post-synaptic neuromuscular blockade (11). It has been used by several investigators (12) for the isolation of nicotinic receptors. Therefore, the distribution of [^{125}I]-α-bungarotoxin binding proteins to the bull sperm fractions was studied.

Methods and Materials. Preparation and characterization of [^{125}I]-α-bungarotoxin. The venom of B. multicinctus was purchased from Sigma Chemical Co. Carrier-free Na [^{125}I] was

purchased from New England Nuclear Corp. Bungarotoxins were separated by chromatography on a CM-Sephadex column according to the procedure described by Lee et al. (13). α-Bungarotoxin (fraction II) and β-bungarotoxin (fraction V) were purified by rechromatography on a CM-cellulose column. Purified α-bungarotoxin was labeled with [^{125}I]iodide by the general procedure of Greenwood and Hunter (14). Radiolabeled α-bungarotoxins were separated from free ^{125}I using a column of Sephadex G-25 and 0.05 M sodium phosphate buffer at pH 7.5. Radiolabeled and diiodo-α-bungarotoxins were fractionated using a column of CM-sephadex C-50, according to the method of Vogel et al. (15).

Mono[^{125}I]-α-bungarotoxin was used in all binding studies. It had a specific activity of 14.45 Ci/mmole. Its pharmacological properties were characterized by its toxicity in mice (LD_{50} 0.38 μg/g), antagonism against ACh at the nicotinic receptors of the frog rectus abdominis muscle (85% depression of the maximal contraction induced by ACh), its binding at the nicotinic receptors of the rat hemidiaphragm and protection of nicotinic receptors of the rat hemidiaphragm against [^{125}I]-α-bungarotoxin binding by nicotine (82% protection at 10^{-3} M).

Preparation of sperm fragments. Ejaculates from the bull were collected and washed with cold calcium-free Krebs-Ringer phosphate solution, as previously described (6). In each experiment, two ejaculates containing in excess of 50 x 10^9 cells were used. Sperm and all solutions were kept at 4°. The washed sperm pellet was resuspended with 12 ml of cold calcium-free Krebs-Ringer phosphate solution and subjected to ultrasonic vibration for 60 sec with a model LS-75 Branson ultrasonic sonifier at a frequency of 20 kilocycles. The head, midpiece and tail fractions were separated by differential centrifugation by a method described by Nelson (7). The fragments obtained of heads, midpieces and tails were estimated by dry smears and light microscopy to be 90-95 percent pure preparations. The protein content of all fragments was determined by the method of Lowry et al. (16). ChA in fractions was analyzed by the method of Sastry and Henderson (17) and AChE by the method of Chiou (18).

Binding of [^{125}I]-α-bungarotoxin to sperm fractions. Sperm fragments, suspended with diluent, were incubated with [^{125}I]-α-bungarotoxin (10^{-8} M) for 1 hr at 37°, vortexing every 10 min. At the end of 1 hr, the samples were centrifuged at 2000 g for 10 min, and the supernatant was discarded. The samples were washed by resuspending with diluent and centrifuging. Washing was continued until the supernatant no longer yielded significant radioactivity. The washed samples were then counted in a Nuclear Chicago gamma counter for 1 min. Binding was reported as dis./min/mg of protein.

Results and Discussion. Isolated sperm fractions of bull spermatozoa were found to bind [^{125}I]-α-bungarotoxin, as shown in Fig. 1. The degree of binding of toxin to tails was higher than that to heads or midpieces. The distribution of the toxin binding is similar to the distribution of ChA and AChE, tail > midpiece > head. This gives further evidence for a nicotinic AChR in sperm, similar to that found in the skeletal muscle.

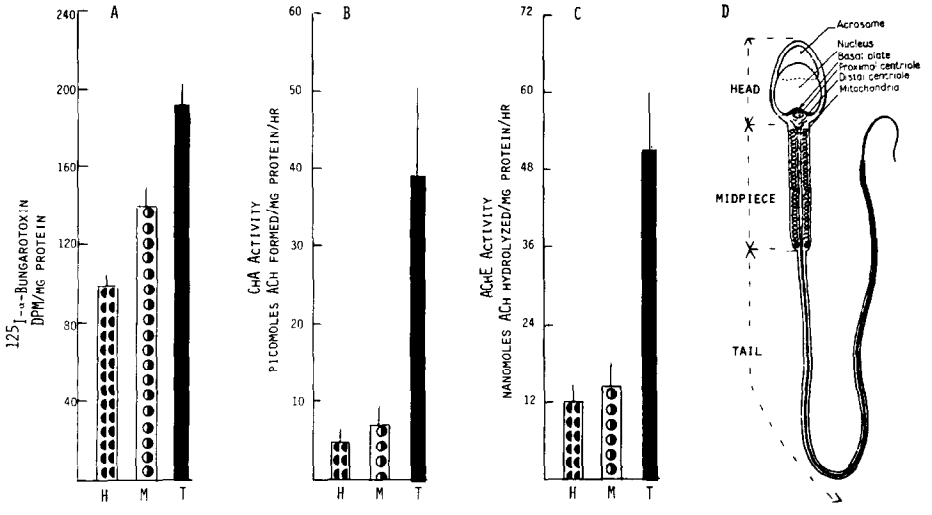

Fig. 1. Distribution of [^{125}I]-α-bungarotoxin (A), choline acetyltransferase (B) and acetylcholinesterase (C) in sperm fractions of heads (H), midpieces (M) and tails (T). Panel D shows the approximate divisions for sperm fractions.

Doses of nicotine (10^{-3} M), d-tubocurarine (5 x 10^{-3} M) and succinylcholine (5 x 10^{-3} M) reduced the [^{125}I]-α-bungarotoxin binding to tails by 82%, 81% and 78% respectively. They reduced toxin binding to heads and midpieces by about 65% and 78% respectively. These drugs, at the above doses, depressed sperm motility by about 80 %.

Specific activities for both ChA and AChE of the tail fraction were about five times higher than the corresponding values for the head. However, the specific activity for [^{125}I]-α-bungarotoxin binding in tails was only twice that in heads. If the nicotinic receptors were to be on the cell surface in bull spermatozoa, as it has been postulated for Arbacia sperm cells, then the nicotinic receptor proteins of the tails would be dislodged more easily than the intracellular enzymes and thereby cross-contaminate the sperm fractions of heads and midpieces. Further work is in progress to evaluate the specificity and the location of [^{125}I]-α-bungarotoxin binding to sperm fractions.

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