An electrophysiological study of N-acetyl-L-asparitic acid (NAAA) on the stellate ganglion of the squid

L. Cecchi, A. De Santis, F. Eusebi and A. Curatolo[1]

II Cattedra di Fisiologia Umana, University of Rome, Città Universitaria, I-00185 Roma, and Stazione Zoologica di Napoli (Italy), 17 April 1978

Summary. The effects of the NAAA have been studied from the giant fibre system of the stellate ganglion of the squid. It suggested that NAAA facilitates the neurotransmitter release by an increase in action potential amplitude of the presynaptic fibres and consequentely by increasing the PSP amplitude.

Briefly, high concentration of NAAA, an aspartate derivative amino acid, is present in the nervous tissue of almost all vertebrates[2]. Conversely, NAAA is reported as undetectable in the central ganglia of invertebrates such as the lobster and horseshoe crab[2]. However, recently, a significant concentration of NAAA (0.73 µmoles/g wet tissue) was isolated from the stellate ganglion of the squid *Loligo vulgaris* by Curatolo et al.[3]. As for direct action on the nervous tissue, NAAA was found not to have any effect on the crayfish stretch receptor[4], or on mammalian spinal cord neurones[5]. Nevertheless, NAAA was found to antagonize the excitatory effects of aspartate or glutamate on cat cerebral cortex through a hyperpolarizing action on the nerve cell membrane[6]. This report describes the action of NAAA on the synaptic transmission of the giant fibre system in the stellate ganglion of the squid.

Materials and methods. The procedure of the dissection was to isolate completely the stellate ganglion[7]. The temperature of the perfusion liquid (natural sea water, pH 8.2, continuously buddled with 100% O_2) was kept at 11 °C. For intracellular recordings with conventional techniques, from the 'main' presynaptic fibre, which forms the giant synapse[8], and from the last giant post-synaptic axon, microelectrodes filled with 3 M KCl were inserted into the fibres at an angle of about 65°. Flexible wire Pt electrodes were used for extracellular stimulation. NAAA was applied by perfusion of the ganglion at a concentration ranging from 5 to 20 mM, in natural sea water (NSW), neutralized with NaOH (pH 8.2). The NAAA was prepared in crystals according to Barker method[9] as its anhydride.

Results. Figure 1 shows 2 examples of the results obtained by perfusing the isolated stellate ganglia with NAAA (10 mM) and by recording the action potential (AP) from the last giant post-axon, evoked by orthodromic extracellular stimulation of the preganglionic nerve at high intensity, in order to activate all the presynaptic fibres[10]. A membrane hyperpolarization (2–3 mV) and an increase of AP size (6–8 mV) on the last axon were observed after a few min of NAAA perfusion. In addition, the synaptic delay and the spike duration decreased, whereas the negative after-potential increased (figure 1). When intracellular stimulation of the 'main' pre-axon has been carried out, only an increase of AP amplitude (3–5 mV) was found (3 experiments). Sometimes the extracellular stimulation of pre-ganglionic nerve did not evoke an orthodromic action potential, but only a synaptic potential uncomplicated by the spike. Perfusing these naturally fatigued preparations (4 experiments) with NAAA (10 mM), a progressive increase of the PSP was found until the spike was evoked (figure 2). The AP amplitude of the 'main' presynaptic fibre increased after the NAAA perfusion (10–11 mV, 4 experiments) according to the membrane hyperpolarization. In the isolated giant post-axon (3 experiments), a membrane hyperpolarization (2–3 mV) like the hyperpolarization in the ganglionic preparation, and consequentely an increase of the AP amplitude (2–3 mV) were observed: this last preparation was assumed as control to discriminate the synaptic effects of NAAA. All the above described effects were reversible and occurred at 5/20 mM NAAA concentration.

Discussion. In the neuromuscular junction of the frog, a

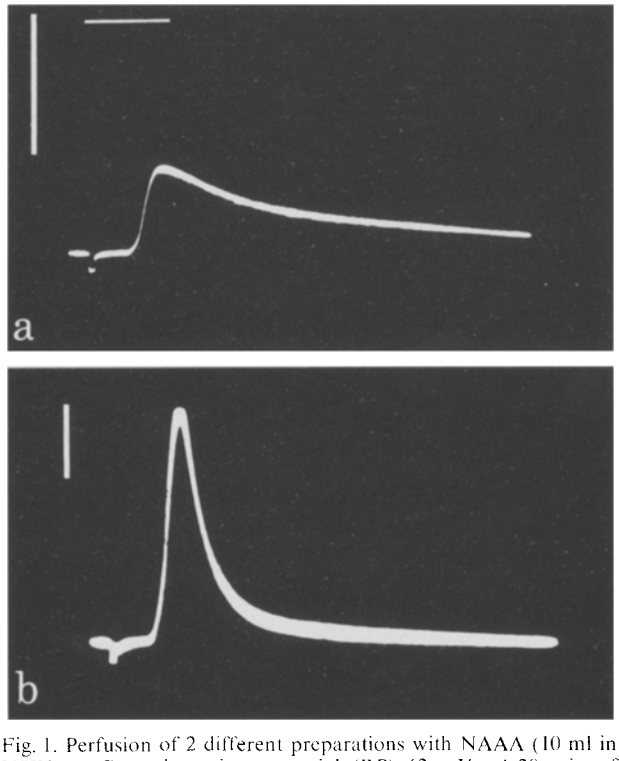

Fig. 1. Perfusion of 2 different preparations with NAAA (10 ml in NSW). *a* Control, resting potential (RP) 62 mV; *a'* 20 min of perfusion: RP 66 mV; *b* control, RP 59 mV; *b'* 25 min of perfusion: RP 63 mV. Intracellular recordings in the last axon and extracellular stimulation of pre-nerve (mantle connective). Horizontal bar: 2.5 msec, vertical bar: 35 mV.

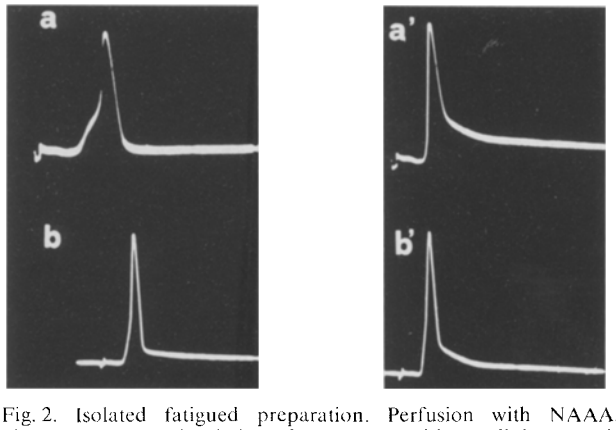

Fig. 2. Isolated fatigued preparation. Perfusion with NAAA, 12 mM in NSW. Stimulation of pre-nerve and intracellular recording in the last giant axon. *a* Control, RP 58 mV; *b* 30 min of perfusion: RP 65 mV. Horizontal bar: 3 msec, vertical bar: 30 mV.

hyperpolarization of the nervous terminal brings about an increase of the neurotransmitter release by the nerve impulses, and an increase of the synaptic potential amplitude[11]. Miledi and Slater[10] showed that it was sufficient to hyperpolarize the membrane of the pre-axon for 8 mV, in order to elicit the spike when a normal EPSP had been depressed to subthreshold level by prolonged repetitive stimulation. These results could shed some light on the effects of NAAA on the synaptic transmission. In fact, the hyperpolarization and consequentely the increase in AP amplitude of the 'main' pre-axon, during NAAA perfusion (mean value of 14 data, 10.5±0.5 mV), presumably produced an increase of the neurotransmitter release by nerve impulses with a consequent increase in AP amplitude of the postsynaptic fibre. In addition the PSP increase in naturally fatigued preparations (figure 2) could be explained by a larger release of neurotransmitter, not only at the giant synapse but also at the proximal synapses. This activation of proximal synapses is supported: a) by the decrease of synaptic delay and shape change of the orthodromic action potential[10]; b) by a relatively smaller increase of the orthodromic AP onset after the intracellular stimulation of the 'main' pre-axon, in respect to that one recorded after the extracellular preganglionic nerve stimulation. Therefore it is suggested that N-acetyl-L-aspartic acid facilitates the neurotransmitter release by a hyperpolarization of the presynaptic fibres, and consequentely by increasing the amplitude of the action potential. As for the membrane potential hyperpolarization observed in the giant fibre system, this effect agrees with previous results described on the mammalian brain[6]. At present, the role of NAAA on the synaptic transmission in the stellate ganglion remains speculative; nevertheless from the above data it is inconsistent with the usually suggested role of NAAA as inert passive anion balancing the high intracellular cation concentration of the nerve cells[2].

1 The authors are indebted with Prof. Ricardo Miledi for his continual advice and suggestions, during various stages of the work. Especial thanks go to Dr C. Betts for her criticism and assistance with the manuscript.
2 H.H. Tallan, J. biol. Chem. *224*, 41 (1957).
3 A. Curatolo, L. Cecchi, F. Eusebi, D. Barra and E. Galli, XXIX Congr. Soc. It. Fisiol., Siena 1977.
4 K.B. Jacobson, J. gen. Physiol. *43*, 323 (1959).
5 D.R. Curtis and J.C. Watkins, J. Neurochem. *6*, 117 (1960).
6 A. Curatolo, M. Marchetti, A. Brancati and A. Salleo, Arch. Sci. Biol. *51*, 98 (1967).
7 T.H. Bullock, J. Neurophysiol. *11*, 343 (1948).
8 J.Z. Young, Phil. Trans. r. Soc. B. *229*, 465 (1939).
9 C.C. Barker, J. chem. Soc. *1953*, 453.
10 R. Miledi and C.R. Slater, J. Physiol. *184*, 473 (1966).
11 J. Del Castillo and B.N. Katz, J. Physiol. *124*, 586 (1954).

Semen electrolytes in normal and infertile subjects. II. Zinc

K.P. Skandhan*, S. Skandhan* and Y.B. Mehta[1]

Department of Physiology, and Department of Pathology, Government Medical College, Surat 395 001 (India), 6 February 1978

Summary. Zinc levels in seminal plasma of normal subjects are compared with those of oligospermic, asthenospermic and azoospermic. A linear direct relationship seems to exist between zinc in seminal plasma and motility of spermatozoans. The possible implications of this are discussed.

The high amount of zinc present in human semen is known since 1921[2]. The importance of this element in semen is not well known. As second in the series[3], we undertook this study to explore the role of zinc in male fertility.

Material and methods. Semen samples, collected after 10 days of abstinence into clean and dry glass bottles, included 37 normal, 34 oligospermic, 9 asthenospermic and 10 azoospermic. The term 'normal' was applied to the samples which fulfilled the requisites in the routine microscopic examination for semen quality and included few from proved fertile persons. Samples with sperm count of less than 40 million/ml were considered as oligospermic and those which showed absence of sperms in deposit despite centrifugation (10,000 rpm × 10) were termed azoospermic. Samples in which sperm motility was less than 50% were grouped as asthenospermic.

Zinc was estimated colorimetrically by the method described by Malmstrom[4]. For convenience we preferred to use iodine flask in place of separatory funnel. Vigorous shaking was done for 5 min. Organic phase was pipetted out with due care.

All necessary precautions were taken to avoid the infiltration of zinc from laboratory wares, distilled water and reagents. The whole procedure was carried out in dust free area.

Results. The motility of normal samples were fair or excellent. In asthenospermic the number of motile sperms varied from 0 to 30%. The results of the present study are given in the table. The values are plotted against the percentage of sperm motility in the figure.

Discussion. There are many reports about the high content of zinc in human semen[5–9], as well as in the semen of animals[10–14]. The values presently obtained in normal subjects (table) are lower than those reported by others[2,6,7]. Climatic difference can change the electrolyte composition of semen[15], and the racial differences and variations in food habits also might be important.

The people of this part of our country (Gujarat) are mostly