GABA-like immunoreactivity in the suboesophageal ganglion of the locust Schistocerca gregaria

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Summary. Neurones in the suboesophageal ganglion of the locust Schistocerca gregaria were stained with an antiserum raised against gamma amino butyric acid (GABA). This ganglion consists of the fused mandibular, maxillary and labial neurones. Immunoreactive cell bodies of similar size and distribution occur in the lateral, ventral and mid-dorsal regions of all three neurones. Approximately 200 cell bodies stain in both the mandibular and maxillary neurones and 270 in the labial neurone. A few distinctly larger cells occur in the ventral groups and one large pair occurs in the lateral group of the maxillary neurone. Dorsal commissures DCIV and DCV are composed mainly of stained fibres, while DCI-DCIII are largely unstained. A ventral commissure also stains in the maxillary neurone. All longitudinal tracts contain both stained and unstained fibres. Many processes within the neuropil are also immunoreactive. A stained axon is found in the posterior tritocerebral commissure which enters the anterior dorsal region of the mandibular neurone. The salivary branch of the 7th nerve contains one stained axon and two axons stain in nerve 8 which innervates neck muscles.

Key words: GABA – Immunohistochemistry – Salivary neurones – Schistocerca gregaria (Insecta)

GABA has been demonstrated quantitatively in the brain, thoracic ganglia and abdominal ganglia of the locust (Breer and Heilgenberg 1985). It is thought to act as an inhibitory transmitter at neuromuscular junctions (Usherwood and Grundfest 1986; Usherwood and Cull-Candy 1975) and in the central nervous system. Immunocytochemical studies using an antibody raised against GABA (Seguela et al. 1984) has allowed the localisation of GABA-like immunoreactivity to groups of neuronal somata, to processes in known tracts and commissures and even to individual identified neurones.

In the brain of the locust, an identified interneurone the tritocerebral dwarf (TCD) (Tyrer et al. 1988) and a population of probable S-neurones whose somata are in the pars intercerebralis adjacent to the ocellar nerve tracts stain positively (Ammermüller and Weiler 1985). About one-third of neuronal somata in the lateral group of cell bodies in the antennal lobe of Manduca are GABA-ergic and some of these are local interneurones (Hoskins et al. 1986). Immunoreactive neurones also occur in the optic lobes of blowflies, houseflies, worker bees and moths (Meyer et al. 1986; Schäfer and Bicker 1986; Homberg et al. 1987), and mushroom body feedback interneurones of the honey-bee are also immunoreactive (Bicker et al. 1985). In the suboesophageal ganglion of Manduca a group of neurones with contralaterally descending axons show GABA-like immunoreactivity (Homberg et al. 1987). In the locust, three pairs of identified inhibitory motor neurones are immunoreactive in each of the three thoracic ganglia (Watson 1986). Additional biochemical (Emson et al. 1974) and pharmacological studies (Usherwood and Cull-Candy 1975) suggest that these neurones may be GABA-ergic. Moreover, in these ganglia, physiologically identified spiking local interneurones that are involved with the processing of mechanosensory inputs from a leg also stain with a GABA antibody (Watson and Burrows 1987). These interneurones (Burrows and Siegler 1982) make inhibitory connections with motor neurones (Burrows and Siegler 1982), with spiking interneurones (Burrows 1987) and with intersegmental interneurones (Laurent 1987). Their inhibitory actions can be blocked by picrotoxin (Watson and Burrows 1987). GABA-like immunoreactivity is first detectable in the neuropil of these thoracic ganglia at 55% development (O'Dell and Watkins 1988). By 65% development the distribution of dorsal and ventral clusters of immunoreactive somata resembles the adult pattern, with the numbers of stained somata reaching adult levels by 85% development. Abdominal ganglia also contain GABA-ergic neurones with somata in 3 clusters, one of which, the medial posterior group is also found in the thoracic ganglia (Watson and Pfüger 1987).

In this paper we complete the description of the general distribution of GABA-like immunoreactivity in the locust central nervous system by examining the suboesophageal ganglion which contains three fused neuromes and provides innervation to the head and neck regions. We show that the staining of groups of cell bodies, tracts and commissures show similarities with those in other segmental ganglia.

Materials and methods

Immunocytochemistry

Adult and first instar Schistocerca gregaria (Forskål) were taken from our crowded culture. The suboesophageal ganglion was fixed in situ with 2.5% glutaraldehyde in 0.05 M phosphate buffer for several minutes, dissected in 0.5% glutaraldehyde (buffered), fixed for 4-6 h in buffered 2.5%
glutaraldehyde, washed for 12–18 h in phosphate buffer, dehydrated and embedded in paraffin wax. Serial sections 10 μm thick were cut and stained using the method of Bishop and O’Shea (1982) which is a modification of the peroxidase/anti-peroxidase method of Sternberger (1974). The GABA antiserum was obtained from Sera Lab and was used at dilution of 1:800. Further details of the method are described by Watson (1986).

**Cobalt filling**

Nerves 7b and 8 were backfilled with cobalt to identify GABAergic cells whose axons run in these nerves. Nerve 7 leaves the posterior labial neuromere ventrolaterally. It divides posterior to the ganglion into branch 7a which innervates the labial submentum and branch 7b which courses posteriorly, laterally and dorsally reaching the salivary duct just anterior to the prothoracic ganglion. N8 branches from a posterior connective, and passes ventrally over the salivary duct to the lateral part of the neck.

The cut end of a particular nerve was immersed in a Vaseline cup containing distilled water for a minute. The water was then replaced by a 3% solution of cobalt chloride. Animals were placed in a moist atmosphere and left overnight. The suboesophageal ganglia were then dissected, placed in ammonium sulphide (Pitman et al. 1972), fixed in buffered formalin for 1 h, and silver intensified (Bacon and Altman 1977). Ganglia were cleared for viewing as wholemounts and then serially sectioned embedded in wax at 10 μm. Sections were counterstained with eosin and drawings made by use of a compound microscope fitted with a drawing tube.

The names of the major commissures and tracts are those used by Tyrer and Gregory (1982).

**Abbreviations:** AC anterior commissure; Md mandibular; Mx maxillary; Lb labial; CON connective; DCI–DCVI dorsal commissures 1–VI; DIT dorsal intermediate tract; DMT dorsal median tract; DVF dorso-ventral fibres; LTD lateral dorsal tract; LVT lateral ventral tract; LF lateral fibres; MVT median ventral tract; RI, 4, 5 roots of nerves 1, 4, 5; SN1 anterior salivary neuron; SN2 posterior salivary neuron; SMC supra-median commissure; TVT transverse fibres; Tr trachea; VC ventral commissure; VF ventral fibres; vcnbT tract containing neurites from ventral cell bodies; VIT ventral intermediate tract; VLT ventral lateral tract; VLMMT ventral lateral mandibular maxillary tract; VMT ventral median tract.

**Stained clusters of somata:** LG lateral group; MDG mid-dorsal group; VG ventral group

**Results**

**Structure of the suboesophageal ganglion**

The basic structure of the suboesophageal ganglion has been described in Tyrer and Gregory 1982. It is composed of three fused neuromeres the mandibular (Md) the maxillary (Mx) and the labial (Lb), and has eight paired nerves (Fig. 1).

The maxillary and particularly the mandibular neuromeres show considerable compression. In the mandibular neuromere, dorsal commissures DCIV and DCV can be seen but the more anterior commissures are not distinguishable. There are three nerves. Nerve 1 (the mandibular) is

![Fig. 1. A Ventral view of the suboesophageal ganglion. The dashed lines represent the boundaries of the mandibular (Md), maxillary (Mx) and labial (Lb) neuromeres. Nerves 1–8 are labelled; B dorsal view to show posterior tritocerebral commissure (PTCC). Branch a contains the tritocerebral giant axon and branch b the tritocerebral dwarf axon. The commissure is drawn larger than scale to show detail. Scale bar: 100 μm](image)

![Fig. 2A, B. Distribution of cell bodies with GABA-like immunoreactivity in the suboesophageal ganglion. A Ventral groups of somata in the adult. The dashed lines indicate the boundaries of the neuromeres. The solid lines 1–14 indicate the levels of sections that are shown as photographs and drawings in Figs. 3–6; B lateral groups (black) and mid-dorsal groups (stippling) of somata in an adult. Scale bar: 100 μm](image)

ventral and innervates the mouthparts (Fig. 1A). Nerve 2 (the hypopharyngeal) is located ventrally near MdDCIII, and nerve 3 is a small dorsal nerve supplying the corpus allatum (Fig. 1B). In the maxillary neuromere all the dorsal commissures can be seen, though it is difficult to distinguish the boundary between MxDCI and MdDCVI. MxVC is present. Only one nerve, N4, is present which is ventral and innervates the maxillary palp (Fig. 1A). The labial neu-