


ON GOING ACTIVITY IN IDENTIFIED NEURONS OF THE RAT SUPERIOR CERVICAL GANGLION BEFORE AND AFTER PARTIAL DENERVATION OF THE SUBMANDIBULAR GLAND

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Ongoing activity was investigated in rat superior cervical ganglion neurons innervating the submandibular gland using intracellular recording techniques. These cells had previously been labelled by fluorescent marker. Ongoing activity was found in 11% of test cells, with a firing rate of 0.1 ± 0.01 Hz. No ongoing activity occurred in the remainder (89% out of 95). Following an experimentally induced reduction in the number of neurons innervating the gland (which had previously been partially denervated), numbers of cells manifesting ongoing activity rose significantly (from 11 to 42%) and ongoing spike rate rose, at 0.3 ± 0.03 Hz. These changes in neurons innervating the partially denervated gland are thought to result from increased convergence on these neurons of influences from preganglionic fibers.

INTRODUCTION

Numerous data obtained from recording the electrical activity of fine filaments or single pre- and postganglionic neurons would point to selective control exerted over peripheral organ activity by the sympathetic nervous system [2-4]. In view of these findings, several workers [3] see the sympathetic ganglia as an accumulation of functionally different and independent groups of neurons via which information from preganglionic neurons is transmitted to peripheral organs. Very little work has yet been done on neuronal connections

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within such functionally homogeneous groups of neurons, however. Findings available in the literature on the convergence on mammalian ganglionic neurons of influences from pregangli-onic fibers with different conduction velocities and excitation thresholds were obtained during work on functionally unidentified neurons [1, 10, 14]. Analysis of naturally occurring activity in functionally identified neurons is therefore an essential step in research under both normal conditions and during experimental procedures, which change the innervation of a given organ.

This study consisted of investigating ongoing activity in superior cervical ganglionic (SCG) neurons innervating the rat submandibular gland following prior denervation and in controls.

METHODS

All electrophysiological experiments were conducted on 300-350 g rats anesthetized by 1.5 g/kg urethane, i.p. An incision was first made on the animal's neck under sterile conditions and the right submandibular gland was freed of connective tissue. Smallish crystals of the fluorescent marker 4-Di-I-ASP [6, 8, 12] with added drops of 2% dimethylsulfoxide solution were placed at several sites between the separated portions of the gland. The gland was then capped with paraflim to provide insulation from the surrounding tissue and the wound was sealed with clips.

Urethane-induced anesthesia of the same animal was repeated 7-10 days later. The rat was fastened to a special platform of a modified fluorescence microscope. A standard operation giving access to the SCG in situ was performed under stereomicroscopic control [10, 11]. An uninterrupted flow of carbogen-saturated standard Ringers solution for warm-blooded animals was supplied to the wound throughout the operation. All connective tissue membranes in the caudal area of the ganglion were removed and by an epifluorescence technique, at a magnification of 300 ×, the site of maximum labelled neuron concentration was found. Neurons innervating the gland were identified by red fluorescence of the marker over the 350-490 μm wavelength (using a rhodamine filter). It should be mentioned that the highest concentration of labeled neurons occurred in the ventrocaudal SCG in all the rat ganglia tested.

Glass microelectrodes filled with a mixture of 1 M KCl solution and 0.5% 5(6)-carboxy-fluorescein was used for recording background electrical activity. Resistance at the tip of these microelectrodes did not exceed 40-50 MΩ. The rhodamine was replaced by a wide bandwidth filter in the microscope to make it easier to penetrate identified neurons, thus also making it possible to keep in sight the visual field of neurons taking up marker at the same time as the microelectrode tip. Electrical activity was recorded on magnetic tape and by pen-writing voltmeter. The same methodological approach was also adopted for rats with preliminary denervation (performed 5 months prior to experimentation) of the right mandibular gland. Methods of partial submandibular gland denervation have already been described elsewhere [12].

RESULTS

Normally Innervated Gland. Intracellular readings were successfully made from 95 ganglionic cells containing fluorescent marker. No ongoing activity at all occurred in 89% of test cells over a minimum observation period of 5 min. These neurons generated full action potentials in response to direct stimulation via an intracellular microelectrode. Ongoing activity, consisting of single, fairly regularly occurring action potentials was observed in only 11% of cells innervating the right mandibular gland (see Fig. 1). Excitatory postsynaptic potentials (EPSP) as well as action potentials arose in the ongoing activity of three out of 10 units. Figure 1 shows samples of ongoing activity recorded from four identified rat ganglion neurons. Mean rate of action potentials in neurons with ongoing activity equalled 0.14 ± 0.01 Hz (n = 10) — approximately the same rate of EPSP occurring in some neurons (= 0.1 Hz). All action potentials had a steep rising front with no deflection of the rising phase (see Fig. 1d), recalling type I ongoing activity in rabbit SCG neurons in this respect [11]. Histograms illustrating intervals in neuronal background firing always showed a preferred interval; distribution of intervals followed a near-normal pattern.

These experiments involved recording activity not just from neurons identified by marker, but also from cells not taking up dye and therefore innervating not the submandibular gland but other peripheral organs. Intracellular readings were taken from 46 non-identified neurons.