MULTIPLE INNERVATION OF AMPHIBIAN SYMPATHETIC GANGLION CELLS

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Intracellular recording was made from B neurons of bullfrog paravertebral ganglia *in situ*. Analysis of the tonically occurring fast e.p.s.p.s revealed that approximately 50 % of B neurons receive a multineuronal innervation and that the rest receive a unineuronal innervation which had been thought to be the characteristic innervation pattern in the amphibian sympathetic ganglia. Similar results were also obtained from the analysis of the fast e.p.s.p.s evoked by stimulation of the spinal nerves containing the spinal B fiber outflows.

INTRODUCTION

As Erulkar and Woodward (1968) have shown with intracellular recording, mammalian sympathetic ganglion cells receive synaptic inputs from several preganglionic fibers differing in conduction velocity. Indeed, these cells in response to a maximal preganglionic stimulus applied away from the ganglion show several peaks of synaptic potential, the number of which decreases as stimulus intensity is reduced stepwise (Erulkar and Woodward, 1968; Nishi, 1974). In case of the amphibian sympathetic ganglion in which the B and C neurons are innervated respectively by preganglionic B and C fibers, only C neurons have been reported to receive multiple innervation (Nishi et al., 1965). Generally, the shape of orthodromic response in B neurons cannot be altered by changing the strength of stimulation applied to the sympathetic chain.

In our recent experiment on bullfrog sympathetic ganglion cells *in situ*, we

have encountered not infrequently the B neurons in which the fast excitatory postsynaptic potentials (fast e.p.s.p.s) due to tonic preganglionic discharges could be divided into several groups depending upon their amplitudes. This observation prompted us to investigate the possibility of multiple innervation of B neurons. The analysis showed that approximately 50 % of B neurons receive synaptic inputs from multiple preganglionic fibers.

METHODS

Bullfrogs (*Rana catesbeiana*) of either sex weighing 380 to 450 g were used throughout. Under a deep ether anesthesia the abdominal cavity was opened and the left or right sympathetic chain was freed from the posterior abdominal wall. The chain was cut below the most caudal ganglion (9th or 10th ganglion) and the central cut-end was led to the dorsal surface through an artificial hole penetrating the lumbar muscle and skin. After the abdomi-

nal opening was tightly closed, the frog was supinely placed in a plaster depression which could accommodate the animal with its all limbs extended. The depression was covered tightly with a plexi-glass plate having a hole of 2.5 cm in radius through which the sympathetic chain could be reached. The cover left almost no space for the frog to move. The lumbar portion of sympathetic chain was gently pulled out and the 9th or 10th ganglion was then led into a small chamber which was clamped in the center of the hole of the cover. The connective tissues surrounding the ganglion were pinned to the Sylgard-filled bottom of the chamber for fixing the ganglion. For insertion of ganglion cells with microelectrodes, only a small area of the capsule covering the ganglion was removed with a great care to minimize injuring the nervous tissues. The sympathetic chain between the frog's dorsum and the chamber was continuously moisten with Ringer and kept as slack as possible so that the movement of the frog would not mechanically disturb the intracellular recording from the ganglion. The ventral and lateral surface of the frog was gently superfused with water at room temperature of 23° to 25°C. This procedure prevented the frog's skin from drying and seemed effective to sedate the animal after its recovery from anesthesia.

The intracellular recording from the ganglion cells was made with conventional microelectrodes filled with 3 M KCl, having tip resistances of 40 to 60 M Ω .

In some experiments, the whole sympathetic chain with the IInd, IIIrd and IVth spinal nerves attached intact was isolated and the responses of B-type neurons in the 9th or 10th ganglion to stimulation of each of the spinal nerves were intracellularly recorded. The me-

thods used for recording and stimulation were similar to those described previously (Nishi and Koketsu, 1960; Nishi et al., 1965).

RESULTS AND DISCUSSION

A majority of ganglion cells showed spontaneous firings at varying frequencies. These firings were mostly orthodromic and elicited by tonic pre-synaptic impulses, but some of them were triggered by injury potentials associated with insertion of the electrode or by antidromic impulses of some unknown origin. A strong hyperpolarization of the cell membrane induced by delivering a constant anodal current abolished both the injury firings and antidromic spikes, while it markedly augmented the fast e.p.s.p.s either subliminal or supraliminal in amplitude. Thus orthodromic responses were readily distinguished from other types of response. Fig. 1 shows a typical recording of spontaneously occurring orthodromic responses of a B neuron obtained during a continued hyperpolarization of the cell membrane to -80 mV. In the figure are seen fast

Fig. 1. Tonicly occurring orthodromic activities of an *in situ* B neuron. The cell membrane was artificially hyperpolarized to -80 mV. Notice the variety of e.p.s.p. amplitudes.

e.p.s.p.s and the e.p.s.p. - triggered action potentials. To our surprise, these e.p.s.p.s showed a variety of amplitudes, and their amplitudes seemed to be classified into three to four groups. In order to analyze the data quantitatively, the amplitude of each e.p.s.p. was corrected for non-linear summation by Martin's method (1955), assuming the equilibrium potential for the fast e.p.s.p. is -10 mV (Nishi and Koketsu, 1960). Fig. 2 shows the histogram of amplitude distribution for a series of 294 e.p.s.p.s of this particular cell. It would be noticed in the histogram that there are four groups of e.p.s.p. amplitude having their peaks at about 1, 10, 20 and 40 mV. The e.p.s.p.s in the smallest group are most likely the spontaneously occurring miniature e.p.s.p.s, whereas the three larger e.p.s.p. groups (I, II and III) would be evoked by tonic presynaptic discharges. This particular ganglion cell, therefore, would be innervated by three presynap-

Fig. 2. Amplitude histogram of tonically occurring e.p.s.p.s.

Fig. 3. Orthodromic responses of B neurons receiving multiple presynaptic innervation.

TABLE 1
Presynaptic innervation pattern of B neurons

No. of fibers innervated	No. of cells (%)	No. of cells receiving input from spinal nerve (II~IV)					
		II	III	IV	II & III	III & IV	II & IV
1	16 (40)	2	1	13			
2	21 (52.5)	2	5	2	2	9	1
3	2 (5.0)	0	0	0	1 2 fibers from III	1 2 fibers from IV	0
4	1 (2.5)	0	0	0	0	1 3 fibers from IV	0

tic fibers, each of which liberated approximately 10, 20 or 40 quanta of transmitter per single impulse. Similar analysis carried out on 33 cells disclosed that 10 cells (30 %) were innervated by a single presynaptic fiber, 18 cells (55 %) by two presynaptic fibers and 5 cells (15 %) by three presynaptic fibers.

We have demonstrated recently that the preganglionic B fibers innervating the B neurons in the 6th through the 10th paravertebral ganglia of bullfrogs outflow from the spinal cord via the IInd, IIIrd and IVth spinal nerves (Yoshimura et al., 1979). We tested, therefore, the responses of B neurons in the 9th ganglion to stimulation of each of the three spinal nerves.

Fig. 3 shows orthodromic responses of two cells receiving multiple innervation. In the cell from which records *a* and *b* were obtained, submaximal and supramaximal stimulation of the IInd spinal nerve induced a single (*a*) and double (*b*) responses, respectively. Stimulation of the IIIrd or IVth spinal nerve did not cause any responses. Obviously this cell was innervated from two preganglionic fibers out-flowing through the IInd spinal nerve. In an-

other cell from which records *c* and *d* were obtained, stimulation of the IInd and IIIrd spinal nerves induced respectively a single (*c*) and double (*d*) e.p.s.p.s, indicating that the cell received one fiber via the IInd spinal nerve and two fibers via the IIIrd spinal nerve.

Table 1 which summarizes the pattern of presynaptic innervation determined by spinal nerve stimulation on 40 B neurons in the 9th ganglion, indicates that 52.5 % of the cells receive two preganglionic fibers, 40 % of them were innervated by a single presynaptic fiber, and the remaining 7.5 % were fed by three to four presynaptic fibers. Of 68 presynaptic fibers innervating the 40 B neurons, the table shows that 32 fibers (47 %) come from the IVth spinal nerve, 26 fibers (38 %) from the IIIrd spinal nerve, and 10 fibers (15 %) from the IInd spinal nerve.

Thus, the present result showed, in contrast to our previous observation, about a half of the lumbar sympathetic ganglion cells receive double presynaptic innervation. This discrepancy might in part be due to the species difference of the animals used; in the previous experiment (Nishi et al., 1965) toads were used instead of bullfrogs. There

were, however, no case of cross innervation with presynaptic C to postsynaptic B unit. All the B neurons examined have received synaptic inputs only from presynaptic B fibers, as we found previously in the toad's ganglia.

It is interesting to note that the average number of presynaptic fibers innervating a single B neuron was 1.9 which was obtained from tonically occurring e.p.s.p.s or 1.7 which was yielded from evoked e.p.s.p.s. The good agreement of the innervation ratios obtained by the two different methods suggests that the majority of presynaptic fibers innervating B neurons are tonically active.

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