Recurrent Synaptic Activation of the Bullfrog Sympathetic Ganglion Cells by Direct Intracellular Stimulation

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Abstract In a certain group (type 2 cells) of bullfrog sympathetic ganglion cells, an action potential exhibiting a triphasic after-potential was produced when the cells were activated by direct intracellular stimulation. This triphasic after-potential consisted of two different potential components, namely, a depolarizing response (DR) and an after-hyperpolarization. The amplitude of DR was increased by increasing the interval between stimuli. Large DRs exceeded the threshold of the cell membrane and produced repetitive firings of spike potentials. The DR was selectively depressed and eventually eliminated in a low-Ca solution. Eserine (10^-5 M) reversibly increased both the amplitude and duration of the DR, and d-tubocurarine reversibly depressed it. These results indicate that the DR of type 2 cells is the EPSP mediated by the nicotinic action of acetylcholine released from preganglionic nerve terminals. Preganglionic nerve fibers innervating type 2 cells are activated through some kind of recurrent pathway formed between them. Recurrent activation of type 2 cells seems to be thus induced when the cells are activated by direct intracellular stimulation.

METHODS

The 9th or 10th lumbar sympathetic ganglia of bullfrog (Rana catesbeiana), isolated with pre- and postganglionic nerve fibres, were used.

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Intracellular recordings of the membrane potential of the ganglion cells were made from cells which were located on the surface of the ganglia by glass capillary microelectrodes filled with 3 M KCl. Tip resistances of microelectrodes were 20–50 MΩ. The methods for recording the membrane potential and stimulating the preganglionic nerve fibres were essentially similar to those described elsewhere (Nishi and Koketsu, 1960).

A ganglion was continuously perfused with either Ringer solution or a test solution. Ionic compositions of solutions used were as follows: Ringer solution (112 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂ and 2.4 mM NaHCO₃; pH, 7.5); low-Ca solution (112 mM NaCl, 2 mM KCl, 0.1 mM CaCl₂, 5 mM MgCl₂ and 2.4 mM NaHCO₃). Drugs used in the present experiments were d-tubocurarine chloride (d-TC) and eserine (physostigmine) sulfate.

All experiments were carried out at room temperature (21–24°C).

RESULTS

Usual response evoked by direct stimulation

Figure 1A shows an action potential associated with a cathodal current pulse applied to the membrane of the ganglion cell through a recording electrode (direct intracellular stimulation) (Nishi and Koketsu, 1960). In this type of action potential a spike potential is followed by a monophasic after-hyperpolarization. The initial peak amplitude of after-hyperpolarizations measured from the resting membrane potential was 20–30 mV (26±1 mV (s.e.), n=10), and the duration ranged from 150–500 msec. Ganglion cells which show this usual kind of action potential are called type 1 cells.

Triphasic after-potential evoked by direct stimulation

In the present experiment, a triphasic after-potential following a spike potential was found when some ganglion cells were activated by direct intracellular stimulation (Fig. 1B). These cells are called type 2 cells.

As seen in Fig. 1B, the configuration of after-potentials was markedly different from that of the type 1 cells (Fig. 1A). In the action potential of type 2 cells,

![Fig. 1. Two types of after-potential following a spike potential evoked by direct stimulation. A: Type 1 cell; action potentials are followed by a monophasic after-hyperpolarization. B: Type 2 cell; triphasic after-potentials follow spike potential. In both cases, the stimulus interval is 5 sec.](image)
the descending phase of the spike reaches a potential level—which is close (about ±10 mV) to the resting membrane potential—within 3 msec, and it swings to a transient depolarizing response (DR). This DR reaches a summit within 9–10 msec and gradually terminates in a tail of after-hyperpolarization.

The DR was never observed when subthreshold stimulations were applied to the cell membrane. The population of type 2 cells was found to be small, i.e., 32 cells out of the 680 cells studied.

**Effect of stimulus interval**

The amplitude of DR varied according to the intervals between stimuli. Four typical responses obtained from a single type 2 cell at different intervals between stimuli are shown in Fig. 2. Each response was produced by the tenth stimulus with different intervals between stimuli, since the amplitude of the DR was gradually reduced during an application of repetitive stimulation and it reached an almost constant value at the tenth stimulation. The peak amplitude of DR was greatly reduced at an interval of 0.25 sec; the value being about +3 mV above the resting potential (Fig. 2-1). When the stimulus interval was increased to 4 sec the amplitude of DR was increased; the value being +15 mV (Fig. 2-2). A further increase in the amplitude of DR at an interval of 30 sec exceeded the threshold of the cell membrane and caused the cell to produce a spike potential (Fig. 2-3). At one minute intervals between stimuli, several repetitive firings of spike potentials (2 to 5 spikes) were initiated by DR (Fig. 2-4).

![Fig. 2. Effect of stimulus interval. Records 1, 2, 3 and 4 were taken at an interval of 0.25, 4, 30 and 60 sec, respectively.](image)

The change in the amplitude of DR caused by different stimulus intervals suggests that some synaptic pathway is involved in the process of generation of DR. Some analyses were therefore made in order to determine whether or not the DR is a postsynaptic potential.
Effect of low-Ca on the DR

When the external solution was changed from the Ringer solution to low-Ca solution the amplitude of the DR decreased and finally disappeared (5 experiments).

Figure 3 illustrates the progressive decrease in the DR in the low-Ca solution. Record 1 is a control response associated with a cathodal current pulse at an interval of one minute. In this case, the DR induced three spike potentials. In the low-Ca solution, the DR decreased progressively and was finally eliminated without a noticeable change in the initial spike potential. The configuration of the after-potential changed from a triphasic to a monophasic wave 5 min after immersion of the ganglion cells in the low-Ca solution (Fig. 3-4). This monophasic after-potential was exactly the same as that of type 1 cells (Fig. 1A). The effect of low-Ca on the DR was rapidly reversed when the ganglion cells were reimmersed in the Ringer solution (Fig. 3-5).

These results suggest that the DR is a postsynaptic potential, since release of a transmitter from the presynaptic terminals is arrested in the low-Ca solution. The fact that a triphasic after-potential is changed to a monophasic after-hyperpolarization in low-Ca solution indicates that the after-hyperpolarization is simply superimposed by the DR.

Effect of d-TC on the DR

The configuration of the DR resembles that of the excitatory postsynaptic potential (EPSP) of ganglion cell (Nishi and Koketsu, 1960). If the DR is the EPSP, a pre-treatment of ganglia with a nicotinic blocking agent, such as d-TC, should prevent initiation of the DR. This possibility was examined in five cells.
Figure 4 shows the effect of d-TC (10^{-5} M) on the DR. Record 1 is a control response produced by a cathodal current pulse in Ringer solution without d-TC. Records 2 to 4 were taken in the presence of d-TC. The amplitude of DR was gradually decreased and eliminated completely 5 min after the application of d-TC. Under these conditions, the action potential was followed by a monophasic after-hyperpolarization (Fig. 4-4), which was exactly the same as that observed in type 1 cells (Fig. 1A). The effect of d-TC was reversible a short time after its removal (Fig. 4-5). These results clearly indicate that the DR and the EPSP are the same.

Effect of eserine on the DR

We expected that the amplitude of DR would be increased in the presence of an anticholinesterase, such as eserine. When eserine (10^{-5} M) was added to the perfusate (the Ringer solution), the amplitude of the DR was increased (5 cells). A typical result is shown in Fig. 5. A notable increase in the amplitude of the DR was observed approximately 5 min after an application of eserine (Fig. 5-2). Such an augmentation of the DR caused repetitive firings of spike potentials (Fig. 5-3, 4). The amplitude of the DR returned to the control value 20–30 min after eserine was removed (Fig. 5-5). These results support the concept that the DR is the EPSP.

Synaptic delay

It is difficult to estimate the exact value of the delay between the initiation of the initial spike and the actual onset of the DR, since the DR overlaps on the descending phase of a spike potential. This value should be smaller than the time difference between the initiation of the action potential and the bottom of the
Fig. 5. Effect of eserine on the DR. Record 1 is the control response in Ringer solution. Records 2 to 4 were taken 5, 10 and 15 min respectively after immersion of the ganglion cells in the solution containing eserine (10⁻⁸ M). The amplitude of DR was markedly increased. Record 5 was taken 20 min after removal of eserine. The horizontal scale indicates 15 msec for records 1, 2, 3 and 5, and 60 msec for record 4.

Fig. 6. Estimation of the synaptic delay. A: The time difference between the initiation of a spike and the onset of a DR is shown by two dotted lines. B: The synaptic delay for the EPSP is shown by the time difference between two dotted lines (see text for details). Time scale; 10 msec for A, 4 msec for B. Voltage scale: 20 mV for A, 2 mV for B. Note the different time scales between A and B.

descending phase of a spike potential (Fig. 6A); the time difference is roughly 3 msec.

Figure 6B shows the synaptic delay for the EPSP of bullfrog sympathetic ganglion. When the preganglionic nerve fibres were stimulated under a condition in which the transmission was blocked to the halves in low-Ca solution, two separate voltage changes could be observed: First, a voltage change generated by the impulse as it arrived in the nerve terminal, and then another (the EPSP) which arose in the postsynaptic membrane. The time difference between the peak of the initial negative voltage change and the onset of EPSP (Fig. 6B) would be the synaptic delay for the EPSP (ECCLES, 1964; KATZ and MILEDI, 1965). The value of this synaptic delay in fourteen cells was 0.96±0.05 msec.
DISCUSSION

The present study demonstrates that the DR following a spike potential is produced in some ganglion cells (type 2 cells) when they are activated by direct intracellular stimulation. According to the present experimental analysis, this DR is identified as the EPSP produced by a nicotinic action of ACh released from preganglionic nerve terminals (Nishi and Koketsu, 1960; Koketsu, 1969). When the interval between stimuli was increased the DR increased in amplitude and it finally produced repetitive firings of spike potentials, as seen in Fig. 2. Since an EPSP is able to initiate only a single action potential of ganglion cells in Ringer solution (cf. Nishi and Koketsu, 1960), observed repetitive firings would be caused by an increase in each DR produced by the preceding spike potential.

The question arises as to why or how the EPSP is produced when an action potential of a type 2 cell is generated. In order to produce the EPSP, preganglionic nerve fibres innervating to type 2 cells must be activated. The possibility that preganglionic nerve fibres are activated by the local current of an action potential of a type 2 cell must be discarded unless some specific electrical coupling (or synapse) between type 2 cells and preganglionic nerve fibres exists, because individual nerve cell elements in neural tissues should be effectively insulated from each other (cf. Hagiwara and Tasaki, 1958). Therefore, the present experimental results suggest that there is some kind of chemical synapse or electrical coupling (or synapse) between type 2 cells and preganglionic nerve fibres.

The delay between the initiation of an action potential and that of the DR was roughly estimated to be less than 3 msec. The synaptic delay for the EPSP from preganglionic nerve endings to ganglion cells was approximately 1.0 msec. Thus, it is possible to assume that an additional recurrent chemical synapse between type 2 cells and preganglionic nerve fibres is involved in the process of the initiation of the DR. Indeed, afferent synaptic connections between preganglionic nerve fibres and ganglion cells have been suggested by morphological and fluorohistochemical studies, particularly in the case of mammalian sympathetic ganglia (cf. Jacobowitz, 1974). If electrical synapses are involved in this process, it seems quite reasonable to assume that there are at least two synaptic steps involved in the process of initiation of the DR.

The physiological significance of such an afferent synapse formed between a type 2 ganglion cell and its presynaptic nerve fibres is not known at the present. Whatever it may be, type 2 cells would produce repetitive firings of action potentials by recurrent activation when a single action potential of these cells is induced by preganglionic activation. It may be noted here that the observed DR is also produced when the preganglionic nerve fibres are stimulated. Such a positive feedback facilitation of postsynaptic neurones may be occurring not only in the sympathetic ganglia but also in the central nervous system.
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REFERENCES


