AN ANALYSIS OF 5-HT HYPERPOLARIZATION OF SYMPATHETIC GANGLION CELLS

Yoshiaki SHIRASAWA and Kyozo KOKETSU*
Tokyo Research Laboratory, Kowa Co., Ltd.
Higashimurayama, Tokyo 189, Japan
*Department of Physiology, Kurume University
School of Medicine, Kurume 830, Japan

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Abstract—Bullfrog sympathetic ganglion cells treated with nicotine are hyperpolarized with application of 5-HT. This 5-HT hyperpolarization, however, was not observed if preparations were pretreated with d-TC before being treated with nicotine. When preparations were treated with ACh or carbamylcholine, which transiently depolarizes ganglion cells, hyperpolarization did take place. Such was also observed in the presence of Ringer's solution if preparations were pretreated with the K+-free Ringer's solution. These results suggested that ganglion cells were hyperpolarized by the action of 5-HT when the Na+-pump of these cells was accelerated by accumulation of intracellular Na+, as the result of a transient depolarization or extracellular K+ deficiency.

As we reported earlier (1), the membrane of bullfrog sympathetic ganglion cells was depolarized with application of 5-hydroxytryptamine (5-HT) in Ringer's solution, whereas it was hyperpolarized by 5-HT when preparations were treated with nicotine. This 5-HT hyperpolarization does not seem to be associated with a blocking action of nicotine on the cholinergic transmission in the sympathetic ganglion, because it is not observed under the effect of d-tubocurarine (d-TC) alone (1). Rather such appears to be associated with a non-specific action of nicotine or with a transient membrane depolarization caused by nicotine.

If a transient membrane depolarization is responsible for a manifestation of the 5-HT hyperpolarization, the 5-HT hyperpolarization should be apparent in Ringer's solution containing acetylcholine (ACh), which causes a transient depolarization of sympathetic ganglion cells, in a manner similar to that seen with nicotine.

We carried out experiments to determine if the sympathetic ganglion cell membrane was hyperpolarized by the action of 5-HT, provided the Na+-pump of these cells was accelerated under the condition where the intracellular Na+ concentration was raised.

MATERIALS AND METHODS

Paravertebral sympathetic ganglion chains from bullfrogs (Rana catesbeiana) were used throughout. Membrane potential changes of ganglion cells were recorded by use of the sucrose-gap method (2). Experimental solutions and their ionic compositions were as follows: Ringer's solution (112 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂ and 2 mM NaHCO₃) and K+-free Ringer's solution (112 mM NaCl, 1.8 mM CaCl₂ and 2 mM NaHCO₃). Drugs
Results

Elimination of the 5-HT hyperpolarization by a treatment with d-TC

A sympathetic ganglion was continuously perfused with Ringer's solution. The ganglion cell membrane was transiently depolarized when nicotine (1.2 x 10^-4 M) was added to Ringer's solution. This depolarization was markedly reduced when ganglia were pretreated with d-TC (10^-4 M) (Fig. 1-1). Under such a condition, the ganglion cell membrane was not hyperpolarized but depolarized by 5-HT (10^-8 M) in spite of the presence of nicotine (Fig. 1-2). After washing off nicotine and d-TC from these preparations, the ganglion cell membrane could be depolarized by a reapplication of nicotine (1.2 x 10^-4 M) (Fig. 1-3). Under this condition, as has been reported in the previous paper (1), the ganglion cell membrane was hyperpolarized by 5-HT (10^-3 M) (Fig. 1-4).

5-HT hyperpolarization after a pretreatment with ACh or carbamylcholine

According to preceding results, it was assumed that the depolarization of sympathetic ganglion cells would be necessary for a production of the 5-HT hyperpolarization. Effects of 5-HT were therefore determined after sympathetic ganglion cells were depolarized by ACh or carbamylcholine.

Sympathetic ganglion cells were markedly depolarized by a high concentration of ACh (5 x 10^-3 M), and this ACh depolarization tended to fall after it reached a maximum amplitude during an application of ACh. Under such an experimental condition, the ganglion cell membrane was hyperpolarized by 5-HT (10^-3 M), as seen in Fig. 2-2. This 5-HT hyperpolarization was markedly inhibited by ouabain (2 x 10^-6 M) (Fig. 2-3), as in the case of the 5-HT hyperpolarization produced in the presence of nicotine (1). The same kind of the hyperpolarization produced by 5-HT (10^-3 M) was observed in the presence of carbamylcholine (3 x 10^-3 M).
According to preceding results, it was assumed that the intracellular accumulation of Na\(^+\) during the membrane depolarization would be responsible for a production of the 5-HT hyperpolarization. Effects of 5-HT were therefore examined under a condition where intracellular Na\(^+\) concentration was increased by soaking preparations in the K\(^+\)-free Ringer's solution.

A sympathetic ganglion was soaked in the cold K\(^+\)-free Ringer's solution (10°C) for 10 hr and perfused with the K\(^+\)-free Ringer's solution (22°C). When the perfusate was switched to the normal Ringer's solution (containing 2 mM K\(^+\)), the ganglion cell membrane hyperpolarized (the K\(^+\)-activated hyperpolarization reported in previous studies (3, 4)) (Fig. 3). Under these experimental conditions, the membrane was again further hyperpolarized by an application of 5-HT (10\(^{-3}\) M) (Fig. 3).

5-HT hyperpolarization in the Na\(^+\)-loaded ganglion cells

According to preceding results, it was assumed that the intracellular accumulation of Na\(^+\) during the membrane depolarization would be responsible for a production of the 5-HT hyperpolarization. Effects of 5-HT were therefore examined under a condition where intracellular Na\(^+\) concentration was increased by soaking preparations in the K\(^+\)-free Ringer's solution.

A sympathetic ganglion was soaked in the cold K\(^+\)-free Ringer's solution (10°C) for 10 hr and perfused with the K\(^+\)-free Ringer's solution (22°C). When the perfusate was switched to the normal Ringer's solution (containing 2 mM K\(^+\)), the ganglion cell membrane hyperpolarized (the K\(^+\)-activated hyperpolarization reported in previous studies (3, 4)) (Fig. 3). Under these experimental conditions, the membrane was again further hyperpolarized by an application of 5-HT (10\(^{-3}\) M) (Fig. 3).

Fig. 3. 5-HT hyperpolarization in a Na\(^+\)-loaded ganglion. The preparation was immersed in the K\(^+\)-free Ringer's solution at 10°C for more than 10 hr and the K\(^+\)-free Ringer's solution was continuously perfused at 22°C. An arrow indicates the time when the perfusate was changed from this solution to Ringer's, and a horizontal line indicates the periods of an application of 5-HT (10\(^{-3}\) M); note the hyperpolarization produced by 5-HT.
The present results indicate that the 5-HT hyperpolarization observed in the presence of nicotine (1) was not associated with a specific action of nicotine but with a transient depolarization caused by nicotine. Furthermore, the results suggest that the 5-HT hyperpolarization was produced when the Na⁺-pump of ganglion cells was accelerated by an accumulation of Na⁺, such being due to a transient depolarization or extracellular K⁺ deficiency.

It has been shown that the 5-HT hyperpolarization is sensitive to ouabain and inhibited in the Na⁺-free Li⁺ solution, suggesting that the 5-HT hyperpolarization is generated by the electrogenic Na⁺-pump (1). Furthermore, it was demonstrated that the K⁺-activated hyperpolarization, which was generated by an activation of the electrogenic Na⁺-pump, was augmented in the presence of 5-HT (4). According to the present experiment, the 5-HT hyperpolarization is produced when the Na⁺-pump of ganglion cells is accelerated. Thus, two possible actions of 5-HT would be proposed. Namely, 5-HT may simply increase the membrane resistance, or alternatively, 5-HT may accelerate the electrogenic Na⁺-pump, particularly when the Na⁺-pump is activated by accumulation of intracellular Na⁺.

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