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PRESYNAPTIC REGULATION OF THE RELEASE OF ACETYLCHOLINE BY 5-HYDROXYTRYPTAMINE

K. HIRAI & K. KOKETSU

Department of Physiology, Kurume University School of Medicine, Kurume 830, Japan

1 The effect of 5-hydroxytryptamine (5-HT) on the release of acetylcholine (ACh) from bullfrog sympathetic preganglionic nerve terminals and frog sciatic nerve terminals was studied with intracellular microelectrodes.

2 The change in transmitter release was measured from the mean quantal content calculated by the variance method from evoked fast e.p.s.ps or e.p.ps in low Ca^{2+}–high Mg^{2+} Ringer solution.

3 5-HT facilitated the release of ACh in low concentrations and depressed it in relatively high concentrations at both preganglionic and motor nerve terminals.

4 These results suggest the possibility that 5-HT may play a role in regulating cholinergic transmission in general.

Introduction

It is known that the release of acetylcholine (ACh) from cholinergic nerve terminals is regulated by catecholamines. However, the mode of this regulation differs for the two kinds of typical cholinergic nerve terminals; the release of ACh from motor nerve terminals is facilitated (cf. Kuba, 1970), whereas that from sympathetic preganglionic nerve terminals is depressed (cf. Christ & Nishi, 1971) by catecholamines. In connection with these actions of catecholamines, the question arises (1) whether 5-hydroxytryptamine (5-HT), a comparable biogenic monoamine, regulates the release of ACh from cholinergic nerve terminals, and if it does, (2) whether the mode of regulation is different for the different cholinergic nerve terminals, as in the case of catecholamines. The present experiments were designed to test whether 5-HT is able to influence the release of ACh from sympathetic preganglionic and motor nerve terminals.

Methods

Ninth or tenth lumbar sympathetic ganglia and sartorius muscles together with sciatic nerves were isolated from bullfrog (*Rana catesbeiana*) and frog (*Rana nigromaculata*), respectively. These preparations were perfused continuously with a Ringer solution and conventional intracellular microelectrode techniques were employed for recording fast excitatory postsynaptic potentials (fast e.p.s.ps) and endplate potentials (e.p.ps) from ganglion and muscle preparations, respectively. Microelectrodes filled with 3 M KCl had tip resistance of 20 to 60 megohm. The ionic composition of Ringer solution was as follows (mM): NaCl 112, KCl 2, $CaCl_2$ 1.8 and $NaHCO_3$ 2.4. Fast e.p.s.ps and e.p.ps were induced by applying preganglionic nerve or motor nerve stimulations at a rate of 0.2 Hz and recorded in a low Ca^{2+}–high Mg^{2+} solution ($CaCl_2$ 0.6 to 1.1 mM; $MgCl_2$ 4.3 to 7.0 mM), the tonicity of which was adjusted by changing the concentration of NaCl. Quantal content was calculated by the variance method (del Castillo & Katz, 1954) from mean amplitude and its standard deviation for 50 to 70 fast e.p.s.ps or e.p.ps. All experiments were carried out at room temperature (20 to 24°C).

The results are presented as means ± s.e. mean; n is the number of observations.

Results

Preparations were continuously perfused with the low Ca^{2+}–high Mg^{2+} solution. When 5-HT in low concentrations (1, 3, 10 or 30 μM) was added to the perfusate, the mean amplitude and mean quantal content of fast e.p.s.ps recorded from ganglion cells were markedly increased, while the resting membrane potential and conductance remained unchanged. The maximum effect was observed with 3 μM. The increases in mean amplitude and quantal content 10 to 15 min after an application of 3 μM 5-HT were 160.3 ± 9.1% (n = 4) and 183.5 ± 6.8% (n = 4), re-

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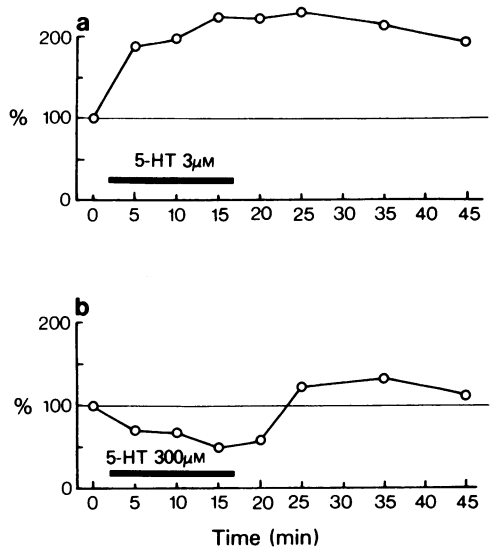

Figure 1 Effects of 5-hydroxytryptamine (5-HT) on the mean quantal content of fast e.p.s.ps recorded from bullfrog sympathetic ganglion. Abscissae show the percentage change in the quantal content of fast e.p.s.ps and each circle indicates the mean quantal content calculated by the variance method. 5-HT at a concentration of 3 μM (a) and 300 μM (b) was added to the perfusate for 15 min as indicated by the bars.

spectively; such a facilitatory effect of 5-HT became smaller when its concentration was increased to 10 to 30 μM. The recovery of preparations from the facilitatory effect of 5-HT was very slow after removal of the drug. Interestingly, when 5-HT in high concentrations (100, 300 or 1000 μM) was added to the perfusate, the mean amplitude and mean quantal content of fast e.p.s.ps were decreased, while the resting membrane potential and conductance remained unchanged. The decrease in the mean amplitude and quantal content by 300 μM 5-HT was 60.7 ± 8.8% ($n = 3$) and 67.0 ± 10.5% ($n = 3$), respectively. The depressant effect of 5-HT in high concentrations disappeared rapidly after the removal of drug. Two typical examples of these results are shown in Figure 1.

Essentially similar dual actions of 5-HT on the release of ACh from the cholinergic nerve terminals were observed with sartorious muscle preparations. Namely, the mean amplitude and mean quantal content of e.p.ps were increased by 5-HT in low concentrations (1, 3 or 10 μM), whereas they were decreased by high concentrations (100 or 300 μM). The maximum increases in the mean amplitude and quantal content were obtained 10 to 15 min after an application of 3 μM 5-HT; they were 136.0 ± 9.9% ($n = 3$) and 174.0 ± 5.5% ($n = 3$), respectively. The decrease in the mean amplitude and quantal content induced by 300 μM 5-HT was 44.1 ± 6.4% ($n = 3$) and 76.3 ± 11.9% ($n = 3$), respectively.

Discussion

The present experiments demonstrated that the release of ACh from sympathetic preganglionic nerve terminals is facilitated by the action of 5-HT in very small concentrations. This was shown by an increase in the quantal content of fast e.p.s.ps. Furthermore, the release of ACh was depressed by 5-HT in relatively high concentrations. This depression was indicated by a decrease in the quantal content of fast e.p.s.ps. Interestingly, essentially similar effects of 5-HT on the release of ACh were obtained from the skeletal muscle preparations. This was in contrast to the action of catecholamines, which depress the release of ACh from sympathetic preganglionic nerve terminals but facilitate that from motor nerve terminals.

The mechanism underlying the concentration-dependent dual action of 5-HT on the evoked release of ACh from both preganglionic and motor nerve terminals is not known at present. A receptor site where 5-HT acts at these cholinergic nerve terminals may be responsible for the manifestation of such a dual action of 5-HT, or, alternatively, two kinds of 5-HT receptors which have different sensitivities or densities at nerve terminals may be responsible. In any case, the fact that the release of ACh from cholinergic nerve terminals can be modified according to the 5-HT concentration (between 10^{-6} and 10^{-5} M) in the extracellular fluid suggests a significant physiological role for 5-HT in the regulation of cholinergic transmission in general.

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