Substance P Facilitates the Release of Acetylcholine from Motor Nerve Terminals of Frogs

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Neurotransmitters can modulate the release of other neurotransmitters from presynaptic nerve terminals, as has been described for "presynaptic inhibition" in the spinal cord (Eccles, 1964). Catecholamine and 5-hydroxytryptamine can alter the release of acetylcholine (ACh) from preganglionic nerve terminals in sympathetic ganglia (Christ and Nishi, 1971; Kuba et al. 1981; Hirai and Koketsu, 1980). Substance P, an endogenous polypeptide, has been considered to be a neurotransmitter candidate for the non-cholinergic slow postsynaptic potential in the mammalian myenteric plexus (Katayama and North, 1978; Johnson et al. 1981), inferior mesenteric ganglia (Konishi et al. 1979; Dun and Jiang, 1982) and bullfrog sympathetic ganglia (Nishi et al. 1980). The present experiments demonstrate that substance P in low concentrations (0.5-4.2 μM) increases the amount of ACh released from motor nerve terminals of the frog neuromuscular junction. It is suggested that substance P might have an important role as a presynaptic modulator of nicotinic cholinergic transmission.

Sartorius muscles with intact sciatic nerves were isolated from frogs (Rana nigromaculata). The preparations were continuously superfused with Ringer solution. Conventional microelectrode techniques were employed for recording the end-plate potential (EPP) using microelectrodes filled with 3 M KCl and having a tip resistance of 20 MΩ. The ionic composition of the Ringer solution was (mM): NaCl 112, KCl 2, CaCl2 1.8 and NaHCO3 2.4. To obtain an appropriate amplitude of EPPs, the sciatic nerves were stimulated at a rate of 0.2 Hz in low Ca2+-high Mg2+ Ringer solutions (CaCl2 0.6-1.1 mM, MgCl2 4.3-7.0 mM) and in Ringer solution containing d-tubocurarine (d-TC) (2 μM).

Quantal content was calculated by the variance method (del Castillo and Katz, 1954) from the mean amplitude and standard deviation of 60 EPPs (5 min) induced before, during and after the application of substance P. Changes in the quantal content during the application of substance P were expressed as percent of the control quantal content.

To obtain the ACh-induced end plate potentials (ACh potentials) and ACh-induced currents (ACh currents), ACh was directly applied to the end-plate by iontophoresis from a microelectrode filled with 1 M ACh with a tip resistance of 70 MΩ. The voltage-clamp methods were similar to
Fig. 1. Effects of substance P (4.2 μM) on the amplitude of the EPP (A) and ACh potentials and ACh currents (B) recorded from frog sartorius muscle end-plates. A: EPPs were induced by stimulation of the sciatic nerve at a rate of 0.2 Hz in Ringer solution containing 2 μM d-TC. Substance P was applied between the downward and upward arrows. B: ACh potentials (records a) and ACh currents (records b) were produced by iontophoretic ACh pulses at a rate of 0.1 Hz. The upper traces indicate the electrical current used for iontophoresis of ACh and the lower traces indicate the ACh potentials or ACh currents.

Fig. 1-A shows the effect of substance P (4.2 μM) on the EPP produced by stimulation of sciatic nerve at a rate of 0.2 Hz. Muscles were superfused with Ringer solution containing 2 μM d-TC to suppress the generation of an action potential. When substance P (4.2 μM) was added to the superfusing solution, the resting membrane potential and resistance (input resistance) did not change significantly. The mean amplitude of the EPPs was increased to 138.6 ± 4.8 % (mean ± S. D., n=4) of the control value by the application of substance P (4.2 μM) for 2 min. A typical result is shown in Fig. 1-A. The augmentation of the EPP was transient; the amplitude of EPP began to recover during the application of substance P (Fig. 1-A). After returning to the control solution, the EPP was restored within 15 min. The facilitatory effect of substance P on the EPP amplitude was concentration-dependent. The minimum effective concentration of substance P was 500 nM.
The effect of substance P on the sensitivity of the nicotinic ACh receptor at the end-plate was examined. The ACh potential induced by microiontophoretic application of ACh to the end-plate was reduced to approximately 73% of the control amplitude by the application of substance P (4.2 μM) for 2 min (Fig. 1-B-a). In 6 preparations, substance P (4.2 μM) reduced the amplitude of the ACh potentials to 75.3 ± 7.1% of the control values. Fig. 1-B-b shows an example of the effect of substance P (4.2 μM) on the ACh currents obtained from a voltage-clamped end-plate. The resting membrane potential was clamped at -80 mV. The amplitude of the ACh current was reduced to 74.7 ± 5.2% (n = 4) of the control amplitude by the application of substance P (4.2 μM) for 2 min.

The mean amplitude of miniature EPPs (MEPPs) recorded in Ringer solution was also decreased by substance P (4.2 μM) from 0.39 ± 0.02 mV to 0.30 ± 0.04 mV. Substance P did not alter the frequency of MEPPs.

These results suggest that the augmentation of the amplitude of the EPPs may be due to a presynaptic facilitatory effect of substance P at the neuromuscular junction. To test this possibility, the effect of substance P on the release of ACh from motor nerve terminals was examined. The quantal content of the EPPs was determined in a low Ca²⁺·high Mg²⁺ Ringer solution. Fig. 2 illustrates the effect of substance P (4.2 μM) on the quantal content of the EPPs from 7 preparations calculated by the variance method. The quantal content slowly but consistently increased during the application of substance P and reached a maximum value within 15 min. The increase of quantal content was maintained for 5 min after returning to the control solution. The quantal content 15

![Fig. 2. Effect of substance P on the quantal content of the EPPs.](Plot.png)
min after beginning the application of substance P (4.2 μM) was 169.2±10.1% of the control. The quantal content of the EPPs returned to control values approximately 30 min after washing out substance P. The facilitatory action of substance P was dependent on the external Ca^{2+} concentration. In Ca^{2+} free Ringer solution, the EPPs were completely eliminated and substance P (4.2 μM) could not restore the EPPs.

A presynaptic facilitatory action of substance P was clearly demonstrated by these experiments. Low concentrations of substance P (0.5–4.2 μM) increased the amount of ACh released from the motor nerve terminals of the frog sartorius muscle. Steinacker (1977) reported that high concentrations of substance P (more than 10 μM) produced an initial depression followed by a long-term augmentation of the amplitude and quantal content of EPPs at the neuromuscular junction of the frog cutaneous pectoris muscle. However, substance P at the concentration used in the present experiments consistently increased ACh release. Therefore the augmentation of ACh-release after removal of a high concentration of substance P (Steinacker, 1977) may correspond to the effect produced by a low concentration of substance P. The present experiments have also demonstrated that the sensitivity of the end-plate was reduced by substance P (Akasu et al. 1983a).

Substance P in low concentrations (0.5–4.2 μM) depressed the after-hyperpolarization of action potentials and prolonged the duration of action potentials in bullfrog sympathetic ganglion cells (Nishimura, 1983). The plateau phase of the Ca^{2+} spike (Minota and Koketsu, 1977) was also prolonged by substance P (Nishimura, 1983). It was suggested (Nishimura, 1983) that substance P may depress the outward currents, I_{K1} (delayed rectifier K^+ current) and I_{K2} (the M current, Brown and Adams, 1980). Therefore micromolar concentration of substance P may increase the amount of ACh released from motor nerve terminals, due to an increase in the Ca^{2+} current during the generation of action potentials in the motor nerve terminal.

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References


