Substance P as a Neuromodulator of Cerebellar Cholinergic Systems

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Substance P in low concentrations (10^{-7} M) activates rat cerebellar neurons (in slices), while in high concentrations (10^{-6} and 10^{-5} M) this compound causes a biphasic response (excitation-inhibition). Substance P probably acts as the excitatory neurotransmitter in the cerebellum and produces modulatory effects (triggering and facilitation) on cerebellar cholinergic structures. Substance P reactivates cholinergic excitatory processes, while acetylcholine prevents substance P-induced inhibitory phase. The data suggest that the modulatory effects of substance P are realized via the feedback mechanisms.

Key Words: *substance P; acetylcholine; neuronal impulse activity; neurotransmitter; neuromodulator*

Substance P (SP) is a peptide neurotransmitter acting both in the central and peripheral nervous systems [4]. It was shown that various brain structures contain SP. High concentrations of SP were found in the substantia nigra, striatum, central gray substance, amygdala, and spinal ganglia. SP is transported along afferent nerve fibers to the posterior spinal roots. Therefore, SP is believed to act as a transmitter in pain processing [7]. SP in low concentrations is present in the hippocampus and cerebellum [2].

SP is a polyfunctional neuropeptide. However, it remains unclear whether SP is a true neurotransmitter or it acts as the neuromodulator in other major neurotransmitter systems (*e.g.*, epinephrine- and cholinergic systems).

Previous studies showed that SP is a neuromodulator in brain cholinergic systems [10,13]. The reciprocal interaction between SP and cholinergic systems was suggested. Destruction of cholinergic structures in the cerebral cortex elevated the content of SP [11]. SP inhibited nicotinic receptors on Renshaw cells of the spinal cord without muscarinic receptor blockade [14]. Cholinergic neurons expressing SP were also found in the gastrointestinal tract (*e.g.*, in the stomach) [15]. However, little is known about functions of SP in various brain structures, in particular, in the cerebellum, and the interaction between SP and cerebellar neurotransmitter systems.

Here we studied the mechanisms underlying *in vitro* effects of SP and the interaction between SP and cerebellar cholinergic neurotransmitter systems.

MATERIALS AND METHODS

Rat cerebellar slices (n=50, 300-500 μ) including the cortex, subcortical and vestibular nuclei were used. After 60-min adaptation in a perfusate (Earle's solution, pH 7.4, 33-34°C) oxygenated with carbogen (95% O_2 and 5% CO_2) neuronal impulse activity (IA) was recorded extracellularly. SP was added in concentrations of 10^{-5}, 10^{-6}, and 10^{-7} M; acetylcholine (ACh) was added in concentrations of 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M.

The results were analyzed by Student's t test.

RESULTS

A well-defined boundary between the cerebellar cortex and white substance corresponding to the Purkinje

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cell (PC) layer allowed us to perform visual control of electrode implantation.

PC demonstrated regular, train, and regular-train activities with firing rates of 3-15 (8.83±1.16), 20-60 (42.85±2.34), and 80-150 spikes/sec (115.70±4.39), respectively.

SP produced a dose-dependent effect. The neuropeptide in a concentration of 10^{-7} M (latency 30-40 sec) increased IA and firing rate of neurons by 2.5-3 times and evoked bursting activity. Apart from low-frequency neurons, there were high-frequency neurons (40-50 spikes/sec) and neurons with the train pattern. Neuronal activation was followed by normalization or slight inhibition of IA and continued upon washout.

SP in high concentrations (10^{-6} and 10^{-5} M) caused a biphasic response. The initial sharp (3-fold) and short-lasting (20-30 sec) rise of neuronal firing rate (Fig. 1) was followed by its drop to 6.60±1.02 spikes/sec ($p<0.01$) and complete inhibition 2.5 min after SP addition.

IA was partially normalized 2-3 min after washout.

Although the cerebellum contains small amounts of SP [2], this neuropeptide was not believed to be the cerebellar transmitter [8]. Therefore, a short-latency response of PC to SP perfused at a rate of 5 ml/min indicates its synaptic properties typical of all transmitters. SP in low concentrations produced excitatory effect probably related to the blockade of the inhibitory neurotransmitter GABA. SP-induced excitation can lead to stable depolarization of nerve cell membranes and secondary inhibition. It was reported that SP fragments induce both the activating and inhibitory effects [5] depending on the state of cell membranes.

The burst response to SP lasted several minutes and was typical of neurons exhibiting train of pulses. The data suggest that SP acts as the neurotransmitter in pacemaker neurons with this firing pattern. Moreover, bursting activity is characteristic of peptide neurons [1].

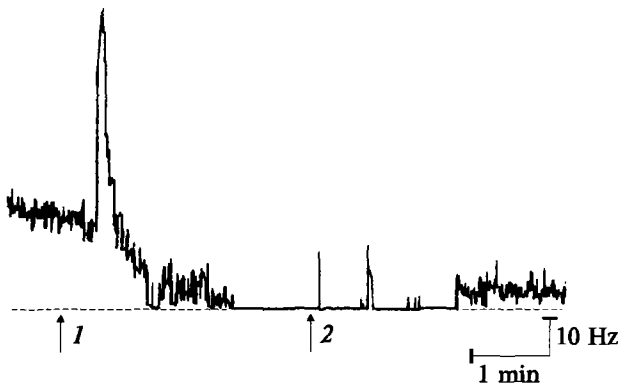

Fig. 1. Effect of substance P (10^{-6} M) of spike frequency of cerebellar neurons (slices): addition (*1*) and washout (*2*). Dotted line: isoline.

Previous data show facilitation-inhibition effects of SP on neurons in the brain nociceptive system [6]. SP is 10,000 times more potent than glutamate in causing depolarization of motor neurons in the ventral horn of the spinal cord. Experiments with tetrodotoxin-induced blockade showed that SP modulates the state of post- and presynaptic membranes. It was hypothesized that the depolarizing effect of SP is due to low K^+ conductance [12]. Compared to other neurotransmitters, SP causes a much more stable depolarization. Prolonged effects of SP are typical of all neuropeptides [4] and associated with the activation of nociceptive-antinociceptive systems [3].

Low doses of ACh (10^{-7} and 10^{-8} M, latency 2.66±0.13 min) caused a 30.4±0.8% increase in neuronal firing rate ($p<0.01$) followed by its spontaneous normalization without washout (Fig. 2). In some cases, these changes were accompanied by the appearance of trains with various interspike intervals. Increasing the concentration of ACh to 10^{-6} and 10^{-5} M progressively decreased and even completely inhibited of IA, which returned to normal after washout.

SP in a concentration of 10^{-6} M was added into the perfusate to study its interaction with ACh. IA in-

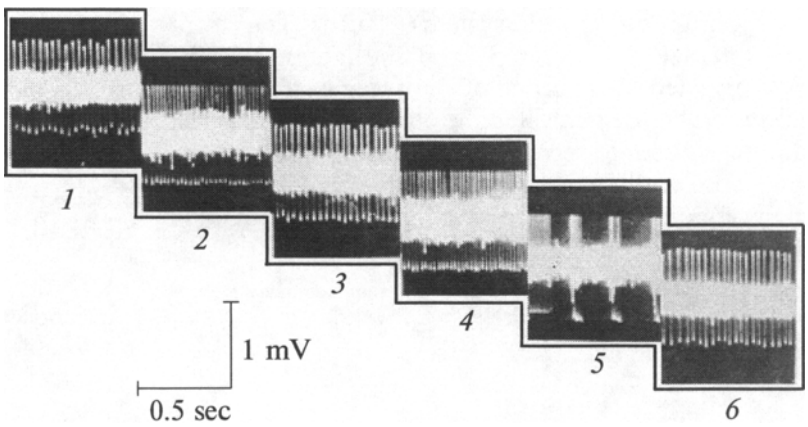

Fig. 2. Impulse activity of a cerebellar neuron in a slice: control (*1*), addition of 10^{-6} acetylcholine (*2*), spontaneous normalization without washout (*3*), addition of 10^{-6} M substance P without washout of acetylcholine (*4, 5*), and normalization after washout (*6*).

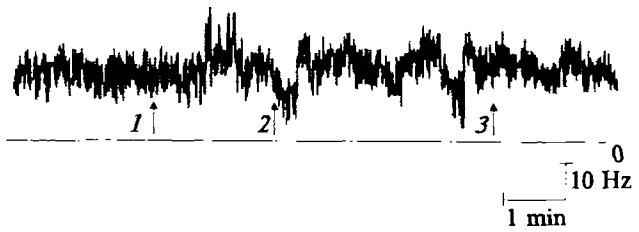

Fig. 3. Spike frequency of cerebellar neurons (slices): addition of substance P (*1*) and acetylcholine (*2*), washout with Earle's solution (*3*), and isoline (*0*).

creased by 30.8±0.9% 40 sec after SP addition (Fig. 3). In a concentration of 10^{-8} M, ACh added at the beginning of SP-induced inhibitory phase (without washout of SP) prevented its progression and normalized IA. Partial inhibition of IA caused by ACh was followed by its spontaneous normalization.

IA increased by 45.70±6.59% ($p<0.01$) after perfusion of slices with Earle's solution containing 10^{-8} M ACh and spontaneously returned to normal, which was probably related to rapid degradation of ACh. The addition of 10^{-6} M SP (without washout, 40 sec latency) increased IA by 59.60±1.79% and evoked train of pulses. In this case, SP did not cause the inhibitory phase. IA returned to normal after washout (Fig. 2).

It can be assumed that SP applied to PC treated with ACh normalized or triggered the cholinergic excitatory mechanism, while ACh introduced at the beginning of SP-induced inhibitory phase prevented its progression. Therefore, SP acts not only as the neurotransmitter, but also modulates the state of cerebellar cholinergic structures. SP increases, decreases, or does not change membrane resistance, which suggests its modulating properties [9]. It was hypothesized that SP activates cerebellar granular cells having cholinergic synapses on mossy fibers, which send projections to the pons, midbrain, and cerebellothalamic tract [8].

Thus, SP plays a role of the excitatory neurotransmitter in cerebellar granular cells and acts as the neuromodulator in cholinergic neurons. SP can block, trigger, or facilitate neuronal activity depending on the initial state of cholinergic structures. It was hypothesized that feedback mechanisms underlie the modulatory effects of SP: the neuropeptide triggers and facilitates activity of cholinergic neurons, while these neurons block the action of SP.

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