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Further support for the postsynaptic action of substance P and its blockade with baclofen in neurons of the guinea-pig hypothalamus in vitro

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Summary. Effects of substance P on neurons of the guinea-pig hypothalamus in vitro and antagonism between substance P and baclofen were investigated. Substance P increased the firing rate of neurons in the medium containing 0 mM Ca^{2+} and 12 mM Mg^{2+}. The excitatory action of stubstance P was antagonized by a low dose of baclofen whereas that of acetylcholine was not antagonized even by much higher doses of baclofen.

There are several lines of evidence to suggest that the undecapeptide, substance P (SP), is involved in synaptic transmission in the brain[2]. As the hypothalamus is one of the areas in the brain rich in SP[3], we investigated the effect of SP on neurons in the hypothalamus of the guinea-pig brain. In addition, we examined the effect of baclofen, a derivative of γ-aminobutyric acid (GABA) and reportedly a specific antagonist of SP[4].

Materials and methods. Adult guinea-pigs of either sex were used. Details of the preparation of the hypothalamic slices (400-600 µm thick), incubation and recording procedures were as described elsewhere[5]. The slices were continuously perfused with Krebs solution, and extracellular unit discharges were recorded with conventional glass microelectrodes. To obtain antidromically as well as orthodromically evoked spikes, an area adjacent to the tip of the recording electrode (about 1 mm distance) was stimulated by single electrical pulses (50 µsec duration) through an electrode consisting of a pair of tungsten wires. After control records were taken, the normal Krebs solution was gradually replaced by the Krebs solution containing the chemical.

Results and discussion. Bath-applied SP markedly increased the spontaneous firing of hypothalamic neurons. Figure 1a illustrates the typical dose-response relations in application of SP in the silent cell of the anterior hypothalamus. To determine whether or not the excitatory action of SP on the hypothalamic neuron was direct, the effect of SP was studied using a Krebs solution containing 12 mM Mg^{2+} but not Ca^{2+}(Ca-free medium). The excitatory action of SP persisted even in the Ca-free medium in 29 out of 30 units tested (see figs. 1b and 3c). Acetylcholine (ACh) in doses of 5.5×10^{-6}–5.5×10^{-8} M also increased the firing rate dose-dependently in both the normal (fig. 1c) and Ca-free (fig. 3a and d) media. All the SP-sensitive neurons were ACh-sensitive, but not for all the ACh-sensitive neurons. As shown in figure 1c, atropine (1.4×10^{-6} M) markedly suppressed the effect of ACh whereas it did not affect the action of SP.

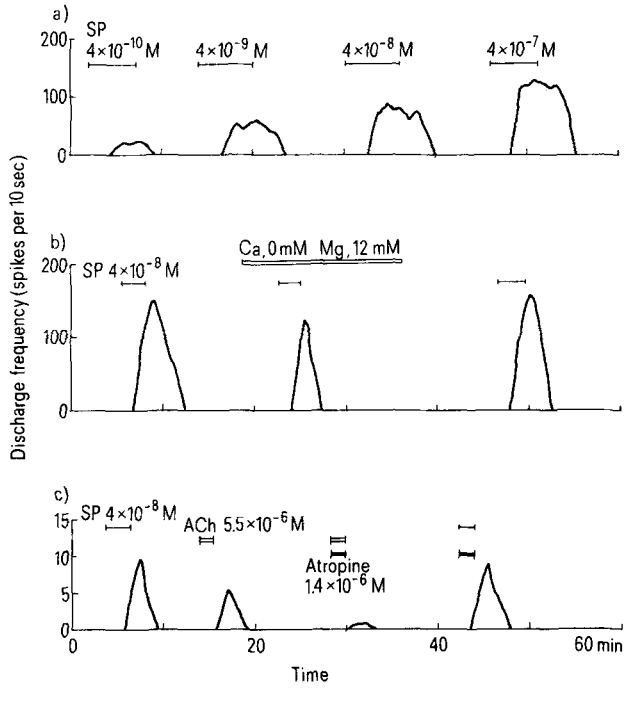

Fig. 1. Effects of substance P (SP) and acetylcholine (ACh) on the spontaneous firing rate of neurons in the anterior hypothalamus. Spontaneous unit discharges were recorded on magnetic tape, digitized by an A-D converter, processed by a general purpose computer Nihon-Kohden ATAC 1200 for compilation of the spontaneous firing rate of the neurons, and recorded on a pen-recorder in this and in subsequent illustrations. Drugs were applied at periods indicated by bars. The bar in *b* entitled 'Ca, 0 mM Mg, 12 mM' represents perfusion with the medium which was calcium free and in which the magnesium was raised to 12 mM. In *c*, antagonism of atropine with ACh is shown.

The effect of baclofen was studied using neurons showing spontaneous discharges. Figure 2 illustrates the typical dose-responses and their time courses obtained in the same neuron with application of baclofen and GABA. Baclofen (8×10^{-8}–8×10^{-7} M) was about 200 times more potent than GABA in suppressing the spontaneous discharge. Baclofen in a dose of 8×10^{-9} M was virtually ineffective in all 10 units tested. As shown in figure 2, both onset and recovery of the baclofen action were apparently slower than those of GABA, regardless of the degree of suppression of the firing rate (note the different time scales in 'perfusion off'). Another distinct difference between the actions of baclofen and GABA was that, in none of 5 units tested did baclofen block antidromic and orthodromic spike generations, even with a high dose of 8×10^{-6} M, whereas GABA in doses of over 2×10^{-4} M consistently blocked both of the evoked spike generations.

As shown in figure 3a, a silent neuron which had started to fire after application of SP in Ca-free medium did not fire when SP was re-applied in combination with baclofen. On the other hand, the excitatory action of ACh was not affected by the same dose of baclofen in any of the 6 units tested (fig. 3a). In separate experiments where a higher dose of baclofen was employed, the action of ACh was not influenced even by baclofen at 8×10^{-6} M in either of the 2 units tested. Figure 3b illustrates the antagonism of SP with baclofen in the spontaneously active neuron. The spontaneous firing rate gradually decreased in the Ca-free medium. When SP was added to the Ca-free medium, the firing rate was markedly increased and remained constant

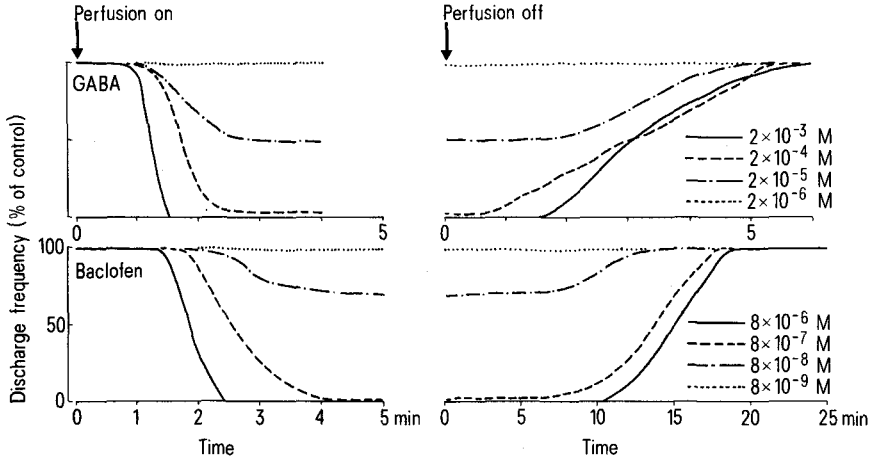

Fig. 2. Effects of baclofen and GABA on the spontaneous firing rate of the spontaneously active neuron in the anterior hypothalamus. All the recordings were made from the same neuron. Firing rates were indicated in the ordinate as % of the control firing rate obtained during perfusion of the standard medium. Note the different time scales in 'perfusion off'.

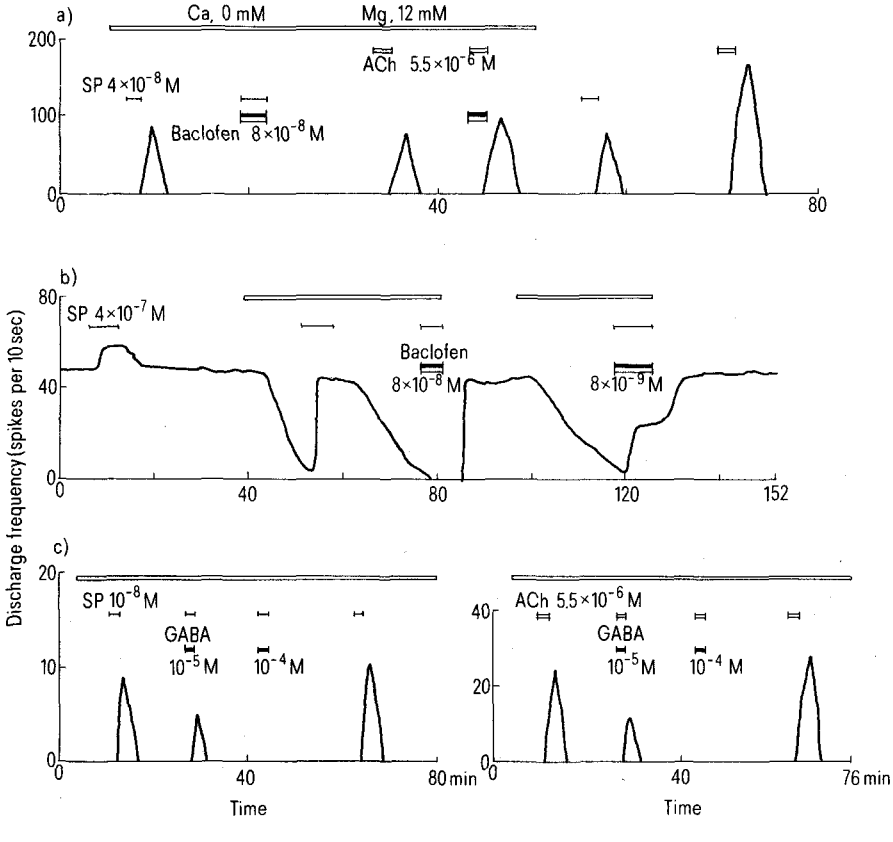

Fig. 3. Effects of baclofen and GABA on the excitatory actions of substance P (SP) and acetylcholine (ACh) on the spontaneous firing rate of neurons in the anterior hypothalamus (a, c and d) and ventromedial hypothalamus (b). Drugs were applied at periods indicated by bars. Bars entitled 'Ca, 0 mM Mg, 12 mM' represent perfusion with the medium which was calcium free and in which the magnesium was raised to 12 mM.

during perfusion of SP. Removal of SP from the medium caused the gradual decrease of the firing rate. When SP was applied again together with baclofen (8×10^{-8} M) under the aforementioned circumstances, the excitatory action of SP was blocked. Introduction of the normal Krebs solution restored the firing rate to the original control level. When SP was applied together with baclofen (8×10^{-9} M) in a Ca-free medium, excitation occurred, but here the magnitude was about half of that produced by SP alone. Namely, the excitatory action of SP in the Ca-free medium was antagonized by a low dose of baclofen which exerted no depressant effect per se. In contrast to baclofen, GABA in a dose of 2×10^{-4} M consistently produced total suppression of the excitations induced by both SP and ACh in the normal Krebs solution (see fig. 3c and d).

Our findings that the excitatory action of SP persisted even in the medium containing 12 mM Mg^{2+} but no Ca^{2+} confirm that SP has a direct action on the postsynaptic membrane in neurons of the guinea-pig hypothalamus, as under these conditions, the possibility of the involvement of a synaptic event can be excluded. This is significant in view of the findings in experiments using the rat spinal cord[6,7] and the guinea-pig sympathetic ganglia[8]. In these experiments, the effect of reduced external Ca^{2+}/Mg^{2+} ratio on the SP-induced excitation was studied using media containing 0.4 mM Ca^{2+} plus 7 mM Mg^{2+}, 0.1 mM Ca^{2+} plus 1.6–3.5 mM Mg^{2+}, and 0.1 mM Ca^{2+} plus 5 mM Mg^{2+}, respectively, owing to the fact that SP-induced excitation was progressively reduced when external Mg^{2+} concentration was increased. Therefore, the possiblity has not been ruled out that the SP-induced excitation in these preparations is mostly due to other excitatory transmitters that are somehow resistant to the lowering of external Ca^{2+} concentration[7].

Baclofen (β-4-chlorophenyl-GABA) is a putative SP antagonist in the central neurons[4]. However, subsequent investigations[9–11] did not confirm the specificity of antagonism between baclofen and SP. This problem has been re-examined by Otsuka and Yanagisawa[7], and they again suggested that baclofen blocks transmission at certain primary afferent synapses by antagonizing the action of SP in the rat spinal cord. On the other hand, Hanley et al.[12] recently reported that baclofen had no effect on ^{3}H-substance P binding in the rat brain membrane. Therefore, the antagonism between SP and baclofen may not be specific. When we placed preparations of the guinea-pig hypothalamus in a Ca-free medium, the action of SP was antagonized by a low dose of baclofen whereas that of ACh was not antagonized even by much higher doses of this drug, and GABA suppressed both excitations induced by SP and ACh. In addition, the time course of the depressant action of baclofen was remarkably slower than that of GABA (fig. 2) suggesting that the action of baclofen is not due to activation of GABA receptors. These findings might indicate that there are some important functional interactions between the actions of SP and baclofen in several portions of the central nervous system, despite the findings of Hanley et al.[12].

1 Acknowledgments. We thank Prof. M. Otsuka for providing baclofen and for helpful suggestions, Prof. H. Kuriyama for valuable discussions, Dr. M. Ohara for critical reading of the manuscript, and Ciba-Geigy (Japan) for providing baclofen.

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Impaired bone resorption of cultured calvaria from mice with abnormal lysosomal function (the Chediak-Higashi syndrome)[1]

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Summary. Spontaneous bone resorption is reduced in cultured calvarial bones from mice with the Chediak-Higashi syndrome, as indicated by decreased mobilization of calcium from the bones to the medium. Although bone resorption in calvaria from mice with this disease can be stimulated by PGE_2 and 1 α $(OH)D_3$, the amounts of mineral released after stimulation is also decreased.

The Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disease which is clinically characterized by increased susceptibility to bacterial infection, pseudoalbinism, pancytopenia, lymphadenopathy, splenomegaly and sometimes lymphoreticular infiltrative disease[2]. The CHS has been described in man, mink, mice, cattle and killer whales and in all these species the occurrence of giant lysosomes in granular neutrophils and monocytes is the hallmark of the disease[2–9]. In the Chediak-Higashi variant of mouse, the beige mouse, the lysosomal anomaly has recently been reported to be manifested also in the osteoclasts[10]. We have shown that CHS mink has increased susceptibility to periodontitis compared to normal range mink, with more advanced periodontal bone destruction and rapid loss of teeth[11]. This observation has been confirmed and extended to the beige mouse[12,13]. Similarily, in patients with CHS, bone loss can be seen already around the teeth in the primary dentition[14].

The increased periodontal bone loss associated with CHS might be due to an inborn error of bone tissue catabolism or be secondary to the increased susceptibility to infection. Although there is a lot of evidence to favour the latter alternative, the first possibility has not been eliminated. We have therefore studied the mineral mobilizing capacity of calvarial bones from the beige mouse and from the corresponding normal wild type (C57BL) in a bone organ culture system.

Material and methods. Calvarial (frontal and parietal) bones from 6 to 7 days old mice, that had been prelabelled with 1.5 µCi ^{45}Ca 4 days prior to sacrifice, were dissected and divided into 2 halves along the sagittal suture. The half-calvaria were separately placed on grids in plastic