24. **High Concentrations of Calcium Prevent the Inhibition of Postsynaptic Potentials and the Accumulation of Cyclic AMP Induced by Adenosine in Brain Slices**

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Adenosine derivatives reversibly cause the inhibition of postsynaptic potentials (PSP),\(^1\) as well as the accumulation of cyclic AMP\(^6\) in the olfactory cortex tissues of guinea pig. Both actions of adenosine are induced extracellularly and remarkably correlate each other.\(^8\) With respect to the inhibitory actions of adenosine on neurotransmissions, Ginsborg and Hirst have shown that adenosine reversibly reduces the quantum content of end-plate potentials and the frequency of miniature end-plate potentials in the phrenic nerve in which transmission was blocked with high Mg\(^2+\) and/or low Ca\(^2+\) concentration.\(^3\) Since it was also proposed that Ca\(^2+\) is involved in the mechanism of transmitter release,\(^5\) for example, for the contraction of the heart the release of transmitter depends on a concentration of Ca\(^2+\) in the nerve terminals,\(^2\) we tested a possibility that adenosine might inhibit the action of Ca\(^2+\) and cause the reduction of transmitter release. The results showed that high concentrations of Ca\(^2+\) in the incubation medium prevented the both effect of adenosine. It is suggested that released adenosine derivatives have important roles in the regulation of synaptic transmission, possibly in a manner of ‘feedback inhibition’, through the control of Ca\(^2+\) movement and the level of cyclic AMP.

Methods. Olfactory cortex slices were prepared from male guinea pigs (300–350 g body weight) by the method of Yamamoto and McIlwaih.\(^16\) Slices were preincubated at 38°C at least for 20 min in the standard Krebs-bicarbonate-glucose medium (glucose 10 mM, NaCl 125 mM, KCl 5 mM, KH\(_2\)PO\(_4\) 1.2 mM, MgSO\(_4\) 1.3 mM, CaCl\(_2\) 1.3 mM and NaHCO\(_3\) 26 mM) gassed with a mixture of 95% O\(_2\) and 5% CO\(_2\), or in the same medium with higher concentrations of Ca\(^2+\) or Mg\(^2+\), made by the addition of appropriate amount of 1.1 M CaCl\(_2\) or 0.77 M MgSO\(_4\). The slice was then transferred to a stimulating and recording chamber and monitored for the amplitude of PSP (N-wave) which were evoked by stimulating the frontal end of olfac-
Olfactory cortex slices were incubated for 5 min with 100 μM adenosine in the medium which contained various concentrations of Ca^{2+} (solid line) or Mg^{2+} (broken line). The bar on each point shows S.E.M. of 3 to 6 experiments.

Fig. 1. Effects of divalent cations on the inhibition of PSP and on the accumulation of cyclic AMP induced by adenosine.

Olfactory trace with rectangular pulses of 10 V in strength and 0.1 msec in duration at 2 cps. After checking the stability of PSP, the original amplitude of the PSP was recorded on an oscilloscope. It increased when Ca^{2+} concentration in the medium was elevated as reported by Richard et al. Then the medium was replaced by a fresh one with the same concentration of Ca^{2+} or Mg^{2+} containing 100 μM adenosine and the slice was further incubated. At 5 min after replaced the
medium, the amplitude of PSP was recorded again and the value of % inhibition was calculated relative to the original amplitude. Immediately after recorded the potential, the slice was homogenized with 1.0 ml of ice cold 6% trichloroacetic acid. The homogenate was centrifuged at 1000 g for 15 min and the amount of cyclic AMP in the supernatant was determined by a modified method of radioimmunoassay15 in which tritiated cyclic AMP was used instead of iodinated one. Protein content of the precipitate was estimated by the method of Miller.10

Results. As shown in Fig. 1, high concentration of Ca²⁺ in the incubation medium prevented the inhibition of PSP and the accumulation of cyclic AMP induced by 100 μM of adenosine. For example, in the medium containing 5.6 mM Ca²⁺, the inhibition of PSP was reduced to 24%, and the accumulation of cyclic AMP was also decreased to 23% of those in the standard medium which contained 1.3 mM Ca²⁺. Both results were statistically significant by Student t test (P<0.01 and P<0.05, respectively). In contrast, when an equivalent amount of Mg²⁺, another divalent cation, was added to the standard medium (concentration of Mg²⁺; 6.0 mM), both effect of adenosine were not significantly changed (P>0.05), comparing with those in the standard medium without the addition (concentration of Mg²⁺; 1.3 mM).

Discussion. Although present results are preliminary, a possible explanation of these in terms of the physiological roles of adenosine derivatives in nervous systems would be as following: (1) Adenosine, AMP, ADP and ATP are released during stimulation accompanied with transmitters from nerve terminal,7 by the exocytosis of synaptic vesicles which contain ATP as well as the transmitter, or by other mechanisms. (2) The released adenosine derivatives inhibit the influx of Ca²⁺ into the nerve terminal and reduce the further release of the transmitter. (3) They also stimulate the accumulation of cyclic AMP which may have relatively long-term effects for the regulation of synaptic transmission; for example, the regulation of enzyme activities, energy supplying systems or memory processes.9

Regulatory roles of adenosine derivatives on neurotransmission in a manner of ‘feed-back inhibition’ may be also popular in the cerebral cortex in which adenosine derivatives depress the electrical activity of many neurons12 as well as increase the level of cyclic AMP in the tissue.14 It is interesting to note that the increase of cyclic AMP can be mediated by the Ca²⁺ movement through Ca²⁺-dependent activator of phosphodiesterases.4

Obviously, there are many other possibilities including that
adenosine derivatives may act by themselves as transmitter\(^1\) in CNS, further studies are necessary to discuss the above hypothesis.

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