Action of GABA and glycine on the membrane potential of cultured astrocytes and the extracellular K^+-concentration

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Summary. The depolarization of cultured astrocytes by GABA and glycine correlates in amplitude and time course with the increase of the extracellular K^+-concentration during perfusion with these amino acids. It is suggested that the glial depolarization is caused by an efflux of K^+ from neighbouring neurones activated by the amino acid transmitters.

From previous studies of the action of amino acid transmitters on cultured glial cells it has been suggested that unlike neurones, glial cells may not possess amino acid receptors and that the depolarization by these neurotransmitters is an indirect effect caused by the efflux of potassium from neighbouring neurones[1-3]. To further test this hypothesis, the extracellular K^+-concentration was measured in nervous tissue cultures during perfusion with GABA or glycine using K^+-sensitive electrodes.

Organotypic cultures were prepared from the lower part of the brain stem and spinal cord of fetal (18-20 days in utero) and newborn rats. The cultures were grown on collagen-coated coverslips either in the Maximov assembly[1]or in roller tubes[4] for 13-35 days. Intracellular recordings were made with glass microelectrodes filled with 4 M K-acetate having resistances between 40 and 150 MΩ (for details Hösli et al.[1,2]). The cultures were constantly perfused with Gey's solution (pH 7.3-7.4) consisting of (mM): NaCl 137, KCl 5, $CaCl_2$ 2.4, $MgCl_2$ 2.2, Na_2HPO_4 1.0, KH_2PO_4 0.18, $NaHCO_3$ 2.9, glucose 11.1.

Measurements of the K^+-concentrations in the bathing fluid in close vicinity of cultured neurones and glial cells were made by using a K^+-sensitive electrode prepared according to the method of Berridge and Schlue[5]. The tips of the siliconized electrodes (diameter 1-2 µm) were filled with a K^+-exchanger resin (Corning 477 317) and the shanks were filled with 0.1 M KCl solution. The resistances of the ion selective electrodes ranged from 2 to 20 GΩ. The electrodes were calibrated in Gey's solution containing 5, 10 and 50 mM K^+ respectively. In the perfusion chamber, electrodes were calibrated in Gey's solution containing 10 mM K^+.

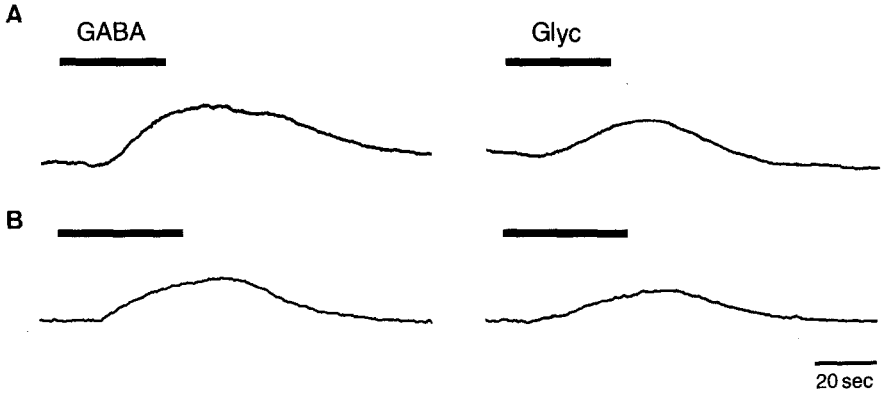
GABA and glycine, which were added to the bathing fluid at a concentration of 10^{-4} M, caused a depolarization of a great number of glial cells without producing significant changes in membrane resistance. The amplitude of the GABA depolarization (mean ± SD: 6.8±3.9 mV, n=79) was usually higher than that produced by glycine (2.7±1.5 mV, n=36). The figure, A, illustrates the depolarizing action of GABA and glycine tested on the same astrocyte in a spinal cord culture.

Perfusion of the cultures with GABA and glycine caused a significant increase in extracellular K^+-activity, the time course being similar to that of the glial depolarization produced by these amino acids. The increase in the K^+-concentration was considerably greater during application of GABA (2.0±0.6 mM, n=19) than of glycine (1.2±0.2 mM, n=12) correlating with the differences in amplitude of the glial depolarization by GABA and glycine respectively (figure, A and B).

A depolarization of astrocytes and an increase of the K^+-concentration were also observed after perfusion with β-alanine and taurine, although the effects of these amino acids were usually smaller than those obtained with GABA and glycine.

Deschenes and Feltz[6] also observed a rise in the K^+-concentration associated with the depolarization of dorsal root ganglion neurones by GABA. Furthermore, during perfusion of the frog spinal cord with GABA and glycine, Kudo and Fukuda[7] found a considerable increase of the extracellular K^+-activity which was accompanied by a depression in spontaneous discharges from the ventral root.

It is concluded that the depolarization of cultured glial cells by GABA and glycine is caused by an efflux of K^+ from adjacent neurones which were hyperpolarized by the amino acid transmitters[8].



A Depolarization of an astrocyte by GABA and glycine. Spinal cord culture, 32 days in vitro. Resting potential - 85 mV. *B* Increase in extracellular K^+-concentration during perfusion with GABA and glycine measured with a K^+-sensitive electrode placed in close vicinity to cultured spinal neurones (culture 17 days in vitro). The rise in K^+ produced by GABA was 1.8 mM and that by glycine 1.2 mM. Duration of perfusion with the amino acids (concentrations 10^{-4} M) is indicated by horizontal bars above tracings.

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