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Short Communication

Activation of β-Adrenergic Receptors Stimulates Taurine Release from Glial Cells

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SUMMARY

1. Activation of adrenergic receptors in primary cultures of astrocytes and LRM55 glial cells stimulates taurine release.

2. Release is inhibited by the β-adrenergic antagonists alprenolol and propanolol but not by the α-adrenergic antagonist phentolamine.

3. Receptor-mediated taurine release occurs via a specific mechanism, since efflux of other amino acids and metabolites is not affected by the β-adrenergic agonist isoproterenol.

The central nervous system is made up of two primary cell types—neurons and glia. Neurons have been described as the primary effectors and glia as passive partners which function to maintain an appropriate metabolic environment for neurons (1). We now present evidence that glia may also play an active role in regulating neuronal activity. The data demonstrate that activation of β-adrenergic receptors on both LRM55 glial cells and primary cultures of astrocytes results in the release of the specific inhibitory neuroactive amino acid taurine. These results not only define a new and dynamic role for glia in central nervous system function, but also indicate that glial cell receptors cannot be ignored in pharmacological studies of the central nervous system.

Taurine release was measured with monolayer cultures of LRM55 glial cells and primary cultures of astrocytes. LRM55 glial cells, a single cell clone obtained from a rat spinal cord tumor, display a number of glial cell properties including high-affinity transport of taurine (2) and glutamate (3), anion transport mechanisms (4), and

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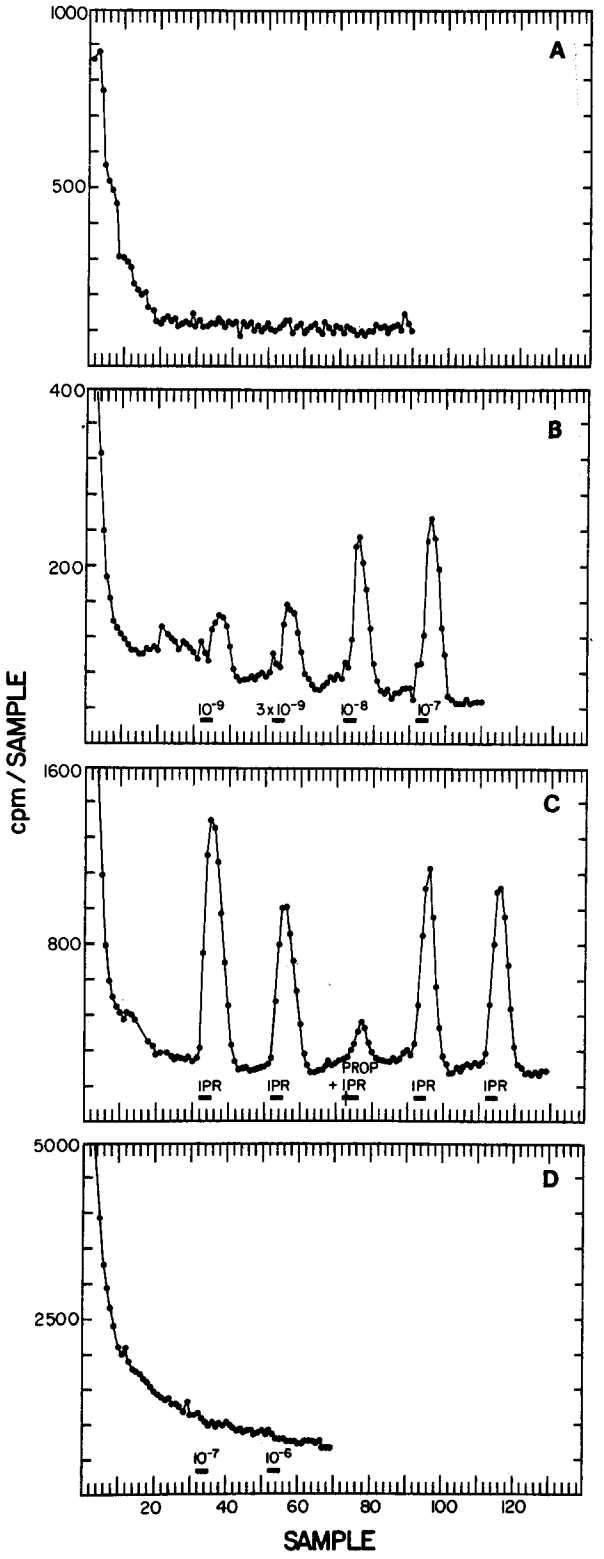
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passive electrical properties (5; unpublished observations). Primary cultures of astrocytes were generously provided by Dr. H. K. Kimelberg, Albany Medical College, Albany, New York, and were prepared by dissociation of newborn rat cerebral cortices (6). For release experiments LRM55 glial cells or primary cultures of astrocytes were grown for 5–10 days on pieces (about 11 × 25 mm) of cell support film (Bellco, Vineland, N.J.). After loading the cells with labeled amino acids, the film was mounted in glass columns and perfused with Hepes-buffered Hanks saline (HBHS; 2).

In our studies of the high-affinity transport of taurine we established that LRM55 glial cells maintain an intracellular taurine concentration of approximately 30 mM, that they can accumulate taurine to concentrations greater than 50 mM, and that they exchange, lose, or metabolize very little of the accumulated taurine (2; unpublished observations). When LRM55 glial cells are perfused continuously with HBHS there is an initial period of rapid efflux followed by a long period of slow efflux. This second period has been followed for more than 120 min and is quite constant; the rate of release is 0.111 ± 0.043%/min at 30 min and 0.073 ± 0.025%/min at 110 min (average ± SD; $N = 22$).

When cells are exposed to the β-adrenergic agonist, (−)-isoproterenol (IPR), there is a dose-dependent increase in the amount of taurine release. Figure 1B presents the raw data of one of several experiments used to generate the dose–response curve observed in Fig. 2 (closed circles). A clear response to IPR was observed at concentrations as low as 10^{-9} M. Receptor-mediated release from LRM55 glial cells can also be stimulated by norepinephrine and epinephrine, although these two naturally occuring agonists are 50- to 100-fold less potent than IPR ($EC_{50} = 2 \times 10^{-7}$, 4×10^{-7}, and 4×10^{-9} M, respectively). Norepinephrine and epinephrine are also 50- to 100-fold less potent than IPR as inhibitors of [^{125}I]iodohydroxybenzylpindolol binding to β-adrenergic receptors on LRM55 glial cells (IC_{50}—2×10^{-3}, 2×10^{-3}, and 5×10^{-5} M, respectively; 7).

Stimulation of taurine release by IPR (10^{-7} M) was reversibly inhibited by propranolol (10^{-8} M), a β-adrenergic antagonist (Fig. 1C). Alprenolol, another β-adrenergic antagonist, also reversibly inhibited IPR-stimulated taurine release. Inhibition of IPR-stimulated taurine release by alprenolol was studied further by measuring the concentration dependence of IPR-stimulated release in the continued presence of alprenolol (10^{-8} M; Fig. 2, filled circles). There is a parallel shift in the IPR dose–response curve to the right ($EC_{50} = 3 \times 10^{-7}$ M), with a maximal response similar to that observed for IPR, suggesting that alprenolol inhibits IPR stimulation by

Fig. 1. Release of [^{3}H]taurine (A–C) or [^{3}H]tryptophan (D) from LRM55 glial cells. Pieces of cell support film with adherent cells were incubated with 10–20 μCi of labeled amino acid (ca. 5×10^{-7} M) in 1 ml of Hepes-buffered Hanks' saline (HBHS) for 30–60 min, transferred to the perfusion apparatus, and perfused at 0.5 ml/min with HBHS without or with added drugs. Fractions were collected at 1-min intervals and the radioactivity in each fraction was determined by liquid scintillation counting. The perfusion medium was changed with a system of valves permitting rapid application and removal of drugs without effecting the rate of perfusion. (A) Efflux from unstimulated cells. (B) Stimulation of taurine release by four concentrations of IPR. The drug was applied at the indicated concentrations for 3 min as shown by the bars. (C) Reversible inhibition of IPR-stimulated release of taurine by propranolol. IPR (10^{-7} M) was applied for 3 min as indicated by the bars. Propranolol (10^{-8} M) was applied for 1 min before and during the third application. (D) Failure of IPR to stimulate tryptophan efflux. Indicated concentrations of the drug were applied for 3 min.

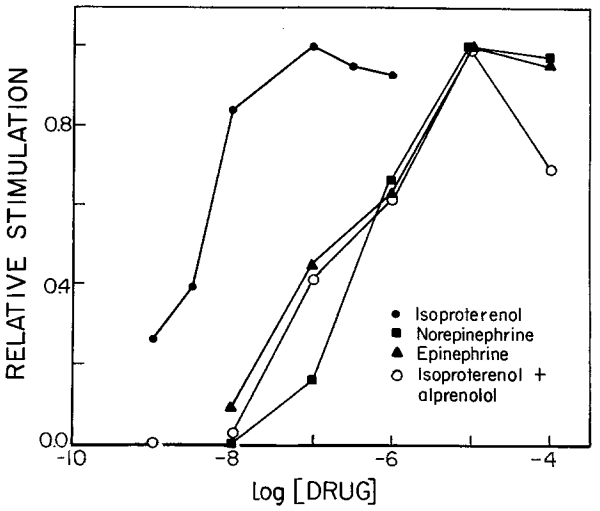

Fig. 2. Stimulation of taurine release as a function of the concentration of IPR (●), norepinephrine (■), epinephrine (▲), and IPR in the presence of 10^{-8} *M* alprenolol (○). To estimate stimulated release the total radioactivity released in response to a drug was calculated by summing the radioactivity in the samples comprising the peak and then subtracting the estimated baseline release. Relative stimulated release at each concentration of a drug was then taken as the fraction of release observed at the concentration of that drug giving maximal release (e.g., 10^{-7} *M* IPR).

competing for the same receptor site. Phentolamine (10^{-4} *M*), an α-adrenergic antagonist, did not inhibit stimulation of taurine release by 10^{-7} *M* IPR, indicating that IPR stimulation of taurine release occurs by activation of β-adrenergic receptors.

To establish that taurine release occurred by a specific mechanism rather than a nonspecific change in cell permeability, LRM55 glial cells were preloaded with [^{3}H]tryptophan and exposed to several high concentrations of IPR (Fig. 1D). No change in [^{3}H]tryptophan efflux was observed. In similar experiments IPR also failed to stimulate release of radiolabeled lysine, urea, or 2-deoxyglucose, thus demonstrating that IPR stimulated release of taurine is not the result of a nonspecific change in cell permeability.

IPR-stimulated release of taurine was also observed with primary astrocyte cultures (Fig. 3), providing strong evidence that receptor-mediated release of taurine is a normal physiological process in astroglia. Taurine release from primary astrocytes was virtually identical to release from LRM55 glial cells. IPR-stimulated release from both cell types was similar and reversibly inhibited by propranolol (cf. Figs. 1C and 3). The baseline (unstimulated) rate of release from primary astrocytes (0.119 ± 0.047 and 0.093 ± 0.049%/min after 30 and 110 min of perfusion, $N = 3$) was indistinguishable from that of LRM55 glial cells. Both cell types generally retained about 80% of the initial radioactivity after 110 min of perfusion.

Neurotransmitter receptors and neurotransmitter stimulation of cyclic AMP synthesis have been described in several glial cell lines (8–14) including LRM55 glial cells (7) and in primary cultures of astrocytes (15–19), but the role of these receptors in

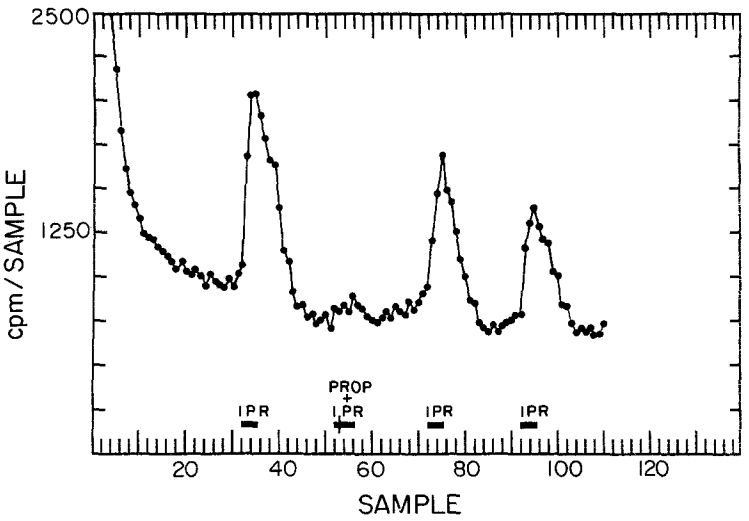

Fig. 3. Stimulation by IPR of [^{3}H]taurine release from primary cultures of astrocytes and its reversible inhibition by propranolol. IPR (10^{-7} *M*) was applied as indicated by the bars. Propranolol (10^{-8} *M*) was applied for 1 min before and during the second application of IPR.

glial cell physiology has not been established. In these experiments we have demonstrated that specific activation of β-adrenergic receptors results in the specific release of taurine from LRM55 glial cells as well as primary cultures of astrocytes, thus describing a potentially important mechanism by which glia may regulate neuronal activity.

Taurine is an unusual neuroactive amino acid. It can represent as much as 50% of the free amino acid pool in rat brain (20), can be accumulated to tissue concentrations as high as 50 m*M* (20,21), and has a long half-life in rat tissues, ≦5 days (22). Taurine causes increases in Cl^- permeability when applied directly on neurons (23,24) and has been shown to have anticonvulsant activity (25–28). Thus the release of taurine by glia may represent a feedback mechanism whereby release of transmitters from neurons, e.g., norepinephrine, stimulates the release of taurine from surrounding glia, consequently decreasing the activity of the same or neighboring neurons.

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