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Noradrenaline- and glutamate-induced taurine release from bulk isolated adult rat astrocytes

Małgorzata Puka[1], **Jan Albrecht**[2] and **Jerzy W. Łazarewicz**[1]

[1]Departments of Neurochemistry and [2]Neuropathology, Medical Research Centre, Polish Academy of Sciences, 3 Dworkowa St. 00-784 Warsaw, Poland

Abstract. The influence of noradrenaline and various agonists of glutamatergic receptors on preloaded [^{3}H]taurine release from bulk isolated adult rat brain astrocytes was investigated by a superfusion technique. In the presence of 1 mM noradrenaline a stimulation of taurine release, resembling that observed in astroglial cultures, was preceded by an inhibition of the efflux, thus demonstrating different dynamics of noradrenaline-evoked taurine release from that observed with ß-agonists on cultured astroglia. Application of 1 mM glutamate and kainate produced stimulation of the release, while 1 mM N-methyl-D-aspartate (NMDA) and 1 mM NMDA together with 65 mM K^+ had no effect on the [^{3}H]taurine release. These data suggest the presence of kainate-sensitive and the absence of NMDA-sensitive glutamate receptors on bulk isolated astrocytes, which is consistent with previous observations on astrocytes in culture.

Short communication

Key words: taurine release, isolated astroglia, adrenergic receptors, glutamatergic receptors, kainic acid, N-methyl-D-aspartate

Taurine (2-aminoethanesulfonic acid) is well known as an inhibitory amino acid in the central nervous system (Huxtable 1989). Its role as a neuro-protective (Schurr et al. 1987) and osmoregulatory (Walz and Allen 1987, Pasantes-Morales and Schousboe 1988, Trachtman et al. 1990) agent has been suggested. However, the mechanism of taurine action in the CNS is not yet clear.

It has been reported that in brain taurine is released into the extracellular space under various pathological conditions, such as ischemia (Benveniste et al. 1984, Hagberg et al. 1985, Puka et al. 1990), neurotoxin action (Lehmann et al. 1985, Lehmann and Hansson 1988, Puka et al. 1990) or hypoosmotic stress (Lehmann 1989, Wade et al. 1988), but the cell compartment and mechanism of its release have not been clarified yet.

Studies on astrocytes in culture revealed that one of the putative sources of taurine may be astroglia (Lehmann and Hansson 1988, Martin et al. 1988, Shain and Martin 1984). However, cultured glial cells have been suspected to express properties that are not normally seen in the CNS (Burnad et al. 1990, MacVicar et al. 1989). We thus assumed that astrocytes bulk isolated from adult brain may be a more adequate model of astroglial cells *in situ*.

In this study, therefore, we assessed the role of noradrenaline and glutamate in the regulation of astroglial taurine release using bulk isolated astrocytes from adult rat brain.

The fraction enriched in astrocytes was bulk isolated from adult rat cerebral cortex with the trypsin-aspiration-differential centrifugation procedure as described previously (Albrecht et al. 1982). The purity of the fraction has been already discussed (Albrecht and Łazarewicz 1990).

Taurine loading and release were carried out as described by Albrecht (1989) for glutamine release. The astrocytes (1 mg protein/ml) were suspended in Krebs-Ringer Tris buffer (140 mM NaCl, 1.3 mM $MgSO_4$, 1.25 mM $CaCl_2$, 5 mM KCl, 1 mM Na_2HPO_4, 10 mM Tris, pH 7.4), preincubated 10 min in 37°C and loaded 30 min with 0.5 mM taurine (Fluka, Switzerland) and [1,2-^{3}H]-taurine (29 mCi/mol, Amersham, UK, final concentration 4 μCi/ml) in 37°C (finally 0.6 mg protein/ml). Then the astrocytes were superfused on Whatman GF/C filters with Krebs-Ringer buffer at a flow rate of 0.5 ml/min for 30 or 40 min to attain constant basal efflux of radioactivity. Fractions were collected every 2 min and radioactivity was counted. Four-minute pulses of various agents were used (noradrenaline, glutamate, kainic acid and N-methyl-D-aspartate, Sigma, USA). Four to six experiments with each compound were performed.

The steady-state of spontaneous taurine efflux was altered by the applied pulses: this effect was expressed as a percentage of extrapolated control efflux.

The taurine concentration used for preloading astroglia (0.5 mM) was previously optimalized in uptake studies. The basal taurine release was not affected by 65 mM K^+ (data not shown).

Noradrenaline (1 mM) produced stimulation of taurine release, maximum 24±8% (mean±SEM, n=6, P<0.05), preceded by prolonged inhibition of the efflux, maximal inhibition 40±5% (n=6, P<0.01) (Fig. 1*NA*).

The stimulation of taurine release was observed with 1 mM glutamate, maximum 19±5% (n=5, P<0.05) (Fig. 1*GLU*). In further studies agonists of two different glutamatergic receptors, NMDA and kainate, were used. The efflux of taurine from astroglia was not influenced by 1 mM NMDA given alone (Fig. 1*NMDA*) or together with 65 mM potassium (data not shown), while it was stimulated by 29±6% (n=4, P<0.05) by 1 mM kainic acid (Fig. 1*KA*).

Taurine release from astroglial cultures has been shown to be stimulated by many neuroactive compounds including noradrenaline, adrenaline, and glutamate (Shain and Martin 1984, Lehmann and Hansson 1988, Martin et al. 1988). However, it appeared reasonable to assume that astrocytes bulk isolated from adult rat brain may react differently (see Introduction).

Noradrenaline applied at 1 mM produced stimulation of taurine release from isolated astroglia (Fig. 1*NA*). This is consistent with the ß-adrenergic receptor-stimulated taurine release from astrocytes in culture observed by others (Martin et al. 1988, Shain and Martin 1984). However, in our experiments performed on isolated cells taurine release was preceded by significant inhibition of the efflux, which was not the case with ß-agonist-induced release in cultured glia (Martin et al. 1988, Shain and Martin 1984). This effect may perhaps be due to initial, transient activation of inward transport of taurine. In support of this concept, Hansson and Rönnbäck (1991) observed an increase of taurine uptake as a result of stimulation of ß-adrenergic receptors in astroglia in primary culture. Detailed studies on the mechanism of noradrenaline-induced taurine release, including description of the second messenger systems, would possibly allow us to explain this phenomenon. It is perhaps noteworthy that a similar transient inhibition of taurine release, although of lesser amplitude, accompanied treatment of astrocytes in culture with aluminum

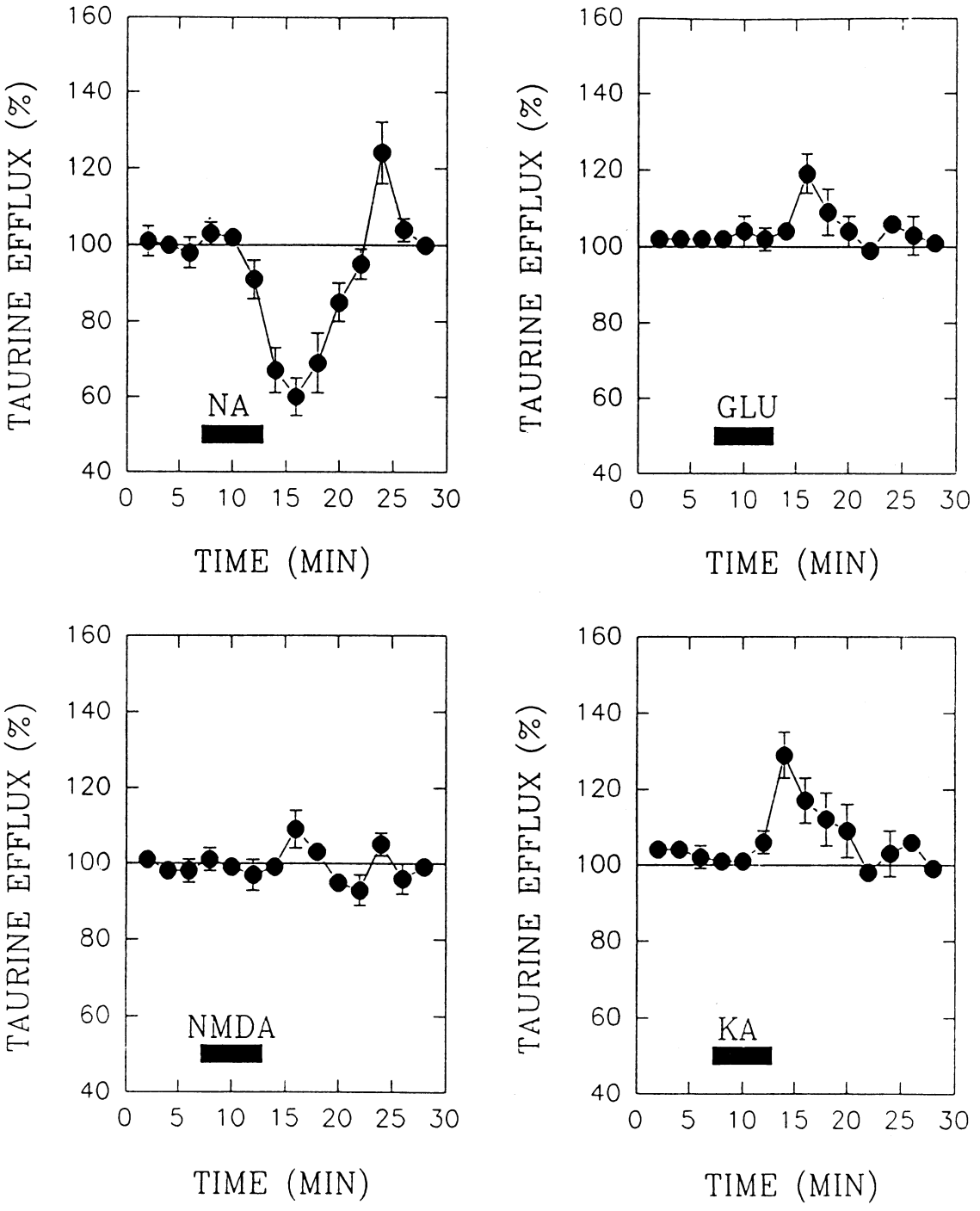

Fig.1. The influence of 1 mM noradrenaline (NA), 1 mM glutamate (GLU), 1 mM N-methyl-D-aspartate (NMDA) and 1 mM kainic acid (KA) on taurine release from bulk isolated rat astrocytes. Horizontal bars indicate 4min pulses of agents. Data were expressed as a percentage of extrapolated level of steady state efflux (mean SEM).

chloride in the absence of sodium chloride (Albrecht and Norenberg 1991).

Stimulation with 1 mM glutamate also produced taurine release from isolated astroglia (Fig. 1*GLU*). It is known from in vivo experiments that stimulation of both kainate- and NMDA-sensitive receptors may induce taurine release into the extracellular compartment (Lehmann et al. 1983, Lehmann et al. 1985, Puka et al. 1990). A role for NMDA receptors in taurine release observed in global cerebral ischemia has also been demonstrated (Puka et al. 1990, Salińska et al. 1991) but the site of release of this amino acid is unclear. Therefore we examined the involvement of these two types of glutamatergic receptors.

The experiments revealed that NMDA did not stimulate taurine efflux in isolated astrocytes even when applied together with a depolarizing concentration of potassium, a procedure known to relieve a voltage-dependent block of NMDA channels by Mg^{2+} (Nowak et al. 1984). On the other hand, application of 1 mM kainic acid significantly stimulated the release of taurine from the astroglia. Very similar data were obtained with astrocytes in culture (Lehmann and Hansson 1988). These results suggest the presence of kainate-sensitive and the absence of NMDA-sensitive glutamatergic receptors on isolated astroglia. This conclusion confirms data obtained in studies on other glutamate-activated processes. For instance, kainate, but not NMDA increases intracellular Ca^{2+} (Pearce et al. 1987, Jensen and Chiu 1990), stimulates polyphosphoinositide metabolism(Pearce et al. 1987, Milani et al. 1989), and produces depolarization of the cell membrane in cultured astrocytes (Kettenmann and Schachner 1985).

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