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Nitric oxide is involved in taurine release in the mouse brain stem under normal and ischemic conditions

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Summary. Nitric oxide (NO) has been shown to regulate neurotransmitter release in the brain; both inhibitory and excitatory effects have been seen. Taurine is essential for the development and survival of neural cells and protects them under cell-damaging conditions. In the brain stem, it regulates many vital functions such as cardiovascular control and arterial blood pressure. Now we studied the effects of the NO-generating compounds hydroxylamine (HA), S-nitroso-N-acetylpenicillamine (SNAP) and sodium nitroprusside (SNP) on the release of preloaded [^{3}H]taurine under normal and ischemic conditions in slices prepared from the mouse brain stem from developing (7-day-old) to young adult (3-month-old) mice. In general, the effects of NO on the release were somewhat complex and difficult to explain, as expected from the multifunctional role of NO in the central nervous system. The basal initial release under normal conditions was enhanced by the NO donors 5 mM HA and 1.0 mM SNAP at both ages, but SNP was inhibitory in developing mice. The release was markedly enhanced by K^+ stimulation. The effects of HA, SNAP and SNP on the basal release were not antagonized by the NO synthase inhibitor N^G-nitro-L-arginine (L-NNA, 1.0 mM), demonstrating that mechanisms other than NO synthesis are involved. Taurine release in developing mice in the presence of SNP was reduced by the inhibitor of soluble guanylate cyclase, 1H-(1,2,3)oxadiazolo(4,3-a)quinoxalin-1-one (ODQ), indicating the possible involvement of cGMP. In normoxia, N-methyl-D-aspartate (NMDA, 1.0 mM) enhanced the SNAP- and HA-evoked taurine release in developing mice and the HA-evoked release in adults. In ischemia, both K^+ stimulation and NMDA potentiated the NO-induced release, particularly in the immature mice, probably without the involvement of the NO synthase or cGMP. The substantial release of taurine in the developing brain stem evoked by NO donors together with NMDA might represent signs of important mechanisms against excitotoxicity which protect the brain stem under cell-damaging conditions.

Keywords: Taurine release – Nitric oxide – Ischemia – Brain stem – Tissue slices – Adult and developing mouse

Abbreviations: cGMP, 3′,5′-cyclic guanosine monophosphate; GABA, γ-aminobutyrate; HA, hydroxylamine; Hepes, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid; L-NNA, N^G-nitro-L-arginine; NMDA, N-methyl-D-aspartate; NO, nitric oxide; NOS, NO synthase; ODQ, 1H-(1,2,4)oxadiazolo(4,3-a)quinoxalin-1-one; SNAP, S-nitroso-N-acetylpenicillamine; SNP, sodium nitroprusside

Introduction

Nitric oxide (NO) has been shown to exert a number of actions as an intercellular messenger or a novel type of transmitter in the brain (Schuman and Madison, 1994). The production of NO is linked to activation of the N-methyl-D-aspartate (NMDA) class of glutamate receptors (Garthwaite, 1991). Synaptically released glutamate acts at postsynaptic NMDA receptors and depolarizes the cell, allowing Ca^{2+} to enter through the receptor-linked ion channels and activating the Ca^{2+}-dependent enzyme NO synthase (NOS) (Bredt et al., 1992). The NO thus formed may either act intracellularly or diffuse out of the cell and act extracellularly at the soluble guanylyl cyclase, enhancing the formation of 3′,5′-cyclic guanosine monophosphate (cGMP) (Knowles et al., 1989). Glutamate participates in the development of ischemic brain injury and NO is therefore likely to be associated with the mechanisms of neuronal damage (Dawson and Dawson, 1996). On the other hand, neurotoxicity is diminished by pharmacological or genetic manipulation of NO production (Izumi et al., 1992; Huang et al., 1994), rendering NO both a neuroprotective and a neurodestructive signal (Iadecola, 1997; Matsui et al., 1999). NO affects neurotransmitter transport in different brain areas, e.g. by evoking release of acetylcholine, monoamines, GABA and glutamate (see Prast and Philippu, 2001, for references). On the other hand, inhibitory effects have been observed in dopamine (Guevara-Guzman et al., 1994), γ-aminobutyrate (GABA) (Getting et al., 1996) and glutamate release (Kamisaki et al., 1995), again a sign of the complex actions of NO.

The inhibitory amino acid taurine has been thought to function as a regulator of neuronal activity, inducing hyperpolarization and inhibiting neuronal firing (Oja and Kontro, 1983a; Saransaari and Oja, 1992). It is also involved in osmoregulation and cell volume adjustments in the central nervous system (Huxtable, 1992). Furthermore, taurine has a special role in immature brain tissue (Oja and Kontro, 1983a; Kontro and Oja, 1987; Sturman, 1993). It is essential for the development and survival of neural cells (see Huxtable, 1992; Sturman, 1993), protects neural cells from excitotoxicity induced by excitatory amino acids in the hippocampus (French et al., 1986), cerebellum (Trenkner, 1990) and neuronal cultures (Tang et al., 1996), prevents harmful metabolic sequences evoked by ischemia or hypoxia (Schurr et al., 1987), and alleviates symptoms in epilepsy (Oja and Kontro, 1983b). We have recently demonstrated that NO-generating compounds modulate taurine release in the adult and developing mouse hippocampus under normal and ischemic conditions, exhibiting both inhibitory and stimulatory effects (Saransaari and Oja, 1999, 2002).

In the brain stem, taurine has been reported to be involved in the modulation of cardiovascular control in the ventrolateral medulla (Kubo et al., 1993; Wang et al., 2005) and in the neurogenic control of arterial pressure in the nucleus of the solitary tract (Meeley et al., 1989). It is released from the locus coeruleus by a variety of stimuli (Singewald and Philippu, 1998) and the released taurine is in this nucleus involved in conditioned fear (Kaehler et al., 2000). On the other hand, also NO has been shown to influence cardiovascular functions and sympathetic nerve activity in the brain stem (Matsuo et al., 2001; Zanzinger, 2002). The levels of taurine in the brain stem are not so high as in the higher brain regions, but for instance the lateral geniculate nucleus, inferior colliculus and auditory brain stem contain taurine in abundance (see Oja and Kontro, 1983a; Palkovits et al., 1986). In two recent papers we have characterized the release mechanisms of preloaded [^{3}H]taurine in slices prepared from the mouse brain stem from developing (7-day-old) an young adult (3-month-old) mice, using a superfusion system (Saransaari and Oja, 2006, 2007). We now completed this series of investigations by studying the properties of taurine release evoked by NO-generating compounds.

Materials and methods

Materials

The Tampere University Committee for Animal Experiments approved the animal experiments. All efforts were made to reduce the number and suffering of the experimental animals. Young adult (3-month-old) and developing (7-day-old) NMRI mice of both sexes were used in the experiments. There were no significant differences in results between the genders. [^{3}H]Taurine (specific radioactivity 1.15 PBq/mol) was obtained from Amersham International, Bristol, UK. All special reagents were from Tocris Bioscience (Northpoint, Avonmouth, UK).

Release experiments

Slices 0.4 mm thick weighing 15–20 mg were manually prepared transversely from the mouse brain stem with a tissue slicer of Stadie-Riggs type. The slices were immediately immersed in 5 ml of oxygenated medium and incubated with 0.01 mM [^{3}H]taurine (50 MBq/l) at 37 °C for 15 min under agitation. The standard medium contained (in mmol/l) NaCl 127, KCl 5, $CaCl_2$ 0.8, $MgSO_4$ 1.3, Na_2HPO_4 1.3, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (Hepes) 15, NaOH 11 and D-glucose 10 (pH 7.4). The slices were then transferred into 0.25 ml cups and superfused with the above medium (unless otherwise specified) at a rate of 0.25 ml/min for a total of 50 min in a system in which freely floating shaken slices were kept under a continuous flow of oxygen in order to preserve their viability (Kontro and Oja, 1987). The superfusion medium was pooled during the first 20 min and thereafter 2-min fractions (0.5 ml) were directly collected into small scintillation vials with a fraction collector. After the 50-min superfusion the slices were weighed, homogenized in ice-cold 5% (w/v) trichloracetic acid solution, and centrifuged, and the clear supernatants were used for scintillation counting. The effluent samples were subjected to the same analyses.

Superfusion conditions

Neural cell damage, called ‘ischemia’, was induced by modified experimental conditions: the slices were superfused in glucose-free medium under N_2 gas from the beginning of the experiments (Taylor et al., 1955).

Estimation of efflux rate constants

Desaturation curves of labeled taurine from the slices were plotted as a function of time on the basis of the radioactivities remaining in the slices after superfusion and recovered in the collected superfusate fractions (Kontro and Oja, 1987). The efflux rate constants of taurine for the time intervals of 20–30 min (k_1) and 32–40 min (k_2) were computed as negative slopes for the regression lines of the logarithm of radioactivity remaining in the slices vs. superfusion time). During the 30–32 min period a new medium replaced the preceding medium in the superfusion chambers for the remaining experimental time until the end of superfusion. The k_1 constants (20–30 min) represent the basal initial release from all slices under normal conditions and in the presence of the NO-generating compounds. The subsequent k_2 constants represent the basal release between 32 and 40 min and the impact of the new effectors added at 30 min. In addition to k_1 and k_2, a third constant k_3 was calculated for the time interval of 40–50 min. These k_3 constants yielded no additional information and are thus not shown.

Statistical calculations

The presence of statistically significant differences between the sample means was detected by variance analysis. Comparison of individual means was made by Hartley’s sequential method of testing.

Results

The effects of NO-generating compounds on taurine release in the brain stem were studied by superfusing the slices with 1.0 mM sodium nitroprusside (SNP), 1.0 mM

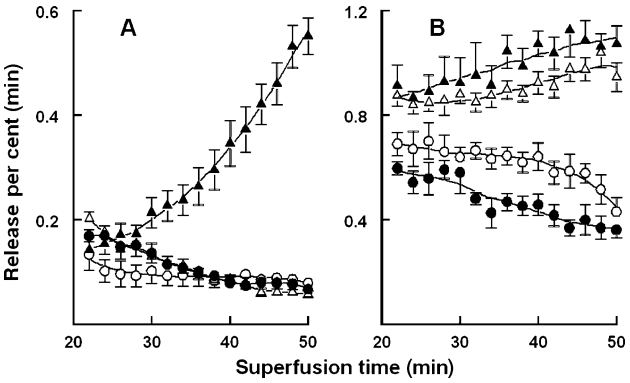

Fig. 1. Effects of 5 mM HA (-▲-), 1.0 mM SNAP (-△-) and 1.0 mM SNP (-○-) on basal taurine release (-●-) from brain stem slices from 7-day-old (**A**) and 3-month-old (**B**) mice in normal conditions. HA, SNAP and SNP were added at the beginning of superfusion. The results are mean values ± SEM of 4–8 independent experiments with SEMs indicated; *HA* hydroxylamine; *SNAP* S-nitroso-N-acetylpenicillamine; *SNP* sodium nitroprusside

S-nitroso-N-acetylpenicillamine (SNAP) or 5.0 mM hydroxylamine (HA) from the beginning of the experiments. Under normal conditions, only HA steeply enhanced taurine release in 7-day-old mice, whereas in adults both HA and SNAP were effective, albeit less markedly (Fig. 1A, B). The effect of HA was concentration-dependent at both ages (Table 1). In all cases, K^+ stimulation (50 mM) enhanced the release markedly more in 7-day-olds than in adults (Table 2). 1H-(1,2,4)Oxadiazolo(4,3-a)quinoxalin-1-one (ODQ, 0.01 mM) applied at 30 min in the presence of SNP reduced taurine release but in the presence of SNAP enhanced it in developing mice, while there were no effects in adults (Table 2). In the presence of SNAP, 1 mM NMDA and ODQ applied at

Table 1. Effects of NOergic compounds on basal initial release from brain stem slices from 7-day-old and 3-month-old mice

Effector (mM)	Efflux rate constants k_1 (20–30 min) ± SEM	
	7-Day-old	3-Month-old
None (control)	0.56 ± 0.03 (47)	2.46 ± 0.06 (76)
SNP 1.0	0.39 ± 0.03** (23)	2.40 ± 0.19 (10)
SNAP 1.0	0.60 ± 0.08 (15)	3.22 ± 0.29** (20)
HA 5.0	0.78 ± 0.07** (43)	3.65 ± 0.29** (19)
HA 10.0	5.52 ± 0.69 ** (8)	6.34 ± 0.44** (8)
L-NNA 1.0	0.51 ± 0.03 (8)	2.85 ± 0.12* (8)

The slices were superfused with the NO-generating compounds from the beginning of the efflux experiments. The results are rate constant k_1 (20–30 min) × 10^{-3} min^{-1} with the number of independent experiments in parenthesis. Significance of differences from the corresponding controls: $^*P<0.05$; $^{**}P<0.01$; *SNP* sodium nitroprusside; *SNAP* S-nitroso-N-acetylpenicillamine; *HA* hydroxylamine; *L-NNA* N^G-nitro-L-arginine

Table 2. Effects of NOergic compounds on taurine release from brain stem slices from 7-day-old and 3-month-old mice under normal conditions

Substance (mM)	Efflux rate constants k_2 ($\times 10^{-3}$ min^{-1}) ± SEM	
	7-Day-old	3-Month-old
None (control)	0.35 ± 0.05 (8)	2.02 ± 0.08 (7)
+50 K^+	1.30 ± 0.09** (8)	2.66 ± 0.09** (7)
SNP 1.0	0.43 ± 0.05 (6)	2.02 ± 0.08 (4)
+50 K^+	0.73 ± 0.09[a] (11)	2.58 ± 0.24 (4)
+1.0 L-NNA	0.37 ± 0.06 (4)	1.97 ± 0.17 (4)
+0.1 NMDA	0.31 ± 0.08 (4)	1.88 ± 0.11 (4)
+0.01 ODQ	0.23 ± 0.01[a] (4)	1.86 ± 0.16 (4)
SNAP 1.0	0.33 ± 0.02 (4)	2.07 ± 0.18 (4)
+50 K^+	1.47 ± 0.38[b] (8)	2.90 ± 0.35 (4)
+1.0 L-NNA	0.39 ± 0.04 (4)	2.66 ± 0.20[a] (6)
+0.1 NMDA	1.39 ± 0.16[b] (6)	2.63 ± 0.30 (7)
+0.01 ODQ	1.07 ± 0.13[b] (6)	2.35 ± 0.22 (4)
HA 5.0	1.23 ± 0.15** (7)	3.04 ± 0.07** (4)
+50 K^+	2.23 ± 0.17[b] (6)	4.33 ± 0.21[b] (7)
+1.0 L-NNA	1.89 ± 0.28 (8)	4.14 ± 0.45 (8)
+0.1 NMDA	2.12 ± 0.13[b] (15)	4.36 ± 0.24[b] (8)
+0.01 ODQ	1.81 ± 0.25 (12)	2.95 ± 0.26 (4)
L-NNA 1.0	0.40 ± 0.04 (4)	2.16 ± 0.12 (4)
+50 K^+	1.33 ± 0.18 (4)	2.74 ± 0.10 (4)

The slices were superfused with the NO-generating compounds from the beginning of the efflux experiments, whereas the other effectors were added at 30 min. The results are rate constants k_2 (32–40 min) × 10^{-3} min^{-1} with the number of independent experiments in parenthesis. Significance of the effects of NO-generating compounds when compared to the corresponding controls: $^*P<0.05$; $^{**}P<0.01$, and the significance of the effects of the other factors when compared to those of the NO-generating compounds: $^aP<0.05$; $^bP<0.01$; *SNP* sodium nitroprusside; *SNAP* S-nitroso-N-acetylpenicillamine; *HA* hydroxylamine; *L-NNA* N^G-nitro-L-arginine; *NMDA* N-methyl-D-aspartate; *ODQ* 1H-(1,2,4)oxadiazolo(4,3-a)quinoxalin-1-one

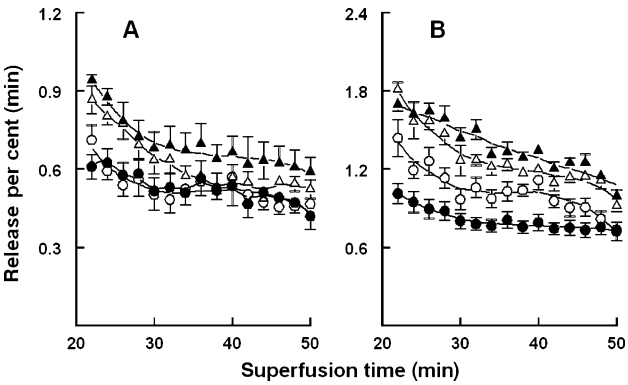

Fig. 2. Effects of 5 mM HA (-▲-), 1.0 mM SNAP (-△-) and 1.0 mM SNP (-○-) on basal taurine release (-●-) from brain stem slices from 7-day-old (**A**) and 3-month-old (**B**) mice in ischemia. HA, SNP and SNAP were added at the beginning of superfusion. The results are mean values ± SEM of 4–8 independent experiments with SEMs indicated; *HA* hydroxylamine; *SNAP* S-nitroso-N-acetylpenicillamine; *SNP* sodium nitroprusside

30 min potentiated the release in 7-day-olds (Table 2). Since the effect of SNAP in adults declined to the normal level by 30 min (Fig. 2A), K^+ stimulation, N^G-nitro-L-arginine (1.0 mM, L-NNA), ODQ and NMDA were applied in some experiments already at the beginning of the superfusion. In these cases the rate constant k_1 was not altered in the presence of L-NNA, ODQ or NMDA, whereas K^+-stimulation significantly ($^{**}P<0.01$) potentiated the rate constants k_1 (20–30 min) (to $6.34 \pm 0.52 \times 10^{-3}$ min^{-1}, $n=4$) and k_2 (to $4.40 \pm 0.26 \times 10^{-3}$ min^{-1}, $n=4$). The HA effect was enhanced by K^+ and NMDA at both ages (Table 2).

Table 3. Effects of NOergic compounds on basal initial taurine release from brain stem slices from 7-day-old and 3-month-old mice in ischemia

Effector (mM)	Efflux rate constants k_1 (20–30 min) ± SEM	
	7-Day-old	3-Month-old
None (control)	1.78 ± 0.12 (27)	4.51 ± 0.21 (15)
SNP 1.0	2.23 ± 0.17* (34)	5.81 ± 0.17** (30)
SNAP 1.0	4.19 ± 0.20** (33)	6.65 ± 0.23** (39)
HA 5.0	4.28 ± 0.25** (26)	7.09 ± 0.16** (35)
L-NNA 1.0	1. 99 ± 0.19 (8)	5.38 ± 0.45 (8)

The slices were superfused under ischemic conditions (under N_2, no glucose) with the NO-generating compounds from the beginning of the efflux experiments. The results are rate constants k_1 (20–30 min) $\times 10^{-3}$ min^{-1} with the number of independent experiments in parenthesis. Significance of differences from the corresponding controls: $^{*}P<0.05$; $^{**}P<0.01$; SNP, sodium nitroprusside; *SNAP* S-nitroso-N-acetylpenicillamine; *HA* hydroxylamine; *L-NNA* N^G-nitro-L-arginine

Table 4. Effects of NOergic compounds on taurine release from brain stem slices from 7-day-old and 3-month-old mice in ischemia

Substance (mM)	Efflux rate constants k_2 ($\times 10^{-3}$ min^{-1}) ± SEM	
	7-Day-old	3-Month-old
None (control)	1.31 ± 0.20 (8)	3.72 ± 0.14 (11)
+50 K^+	3.35 ± 0.25** (8)	4.55 ± 0.30* (8)
SNP 1.0	1.95 ± 0.15* (8)	4.55 ± 0.37* (8)
+50 K^+	3.30 ± 0.25[b] (8)	5.55 ± 0.28[a] (8)
+1.0 L-NNA	3.02 ± 0.37[b] (8)	5.05 ± 0.39 (7)
+0.1 NMDA	2.66 ± 0.54[b] (6)	4.84 ± 0.24 (4)
+0.01 ODG	2.30 ± 0.35[b] (4)	3.89 ± 0.28 (4)
SNAP 1.0	3.98 ± 0.22** (8)	5.26 ± 0.17** (8)
+50 K^+	5.08 ± 0.33[b] (5)	6.17 ± 0.36[a] (8)
+1.0 L-NNA	4.59 ± 0.50 (8)	5.34 ± 0.34 (8)
+0.1 NMDA	4.71 ± 0.32[b] (12)	6.84 ± 0.50[b] (4)
+0.01 ODG	5.60 ± 0.64[b] (4)	5.37 ± 0.25 (4)
HA 5.0	4.33 ± 0.21** (4)	5.73 ± 0.22** (8)
+50 K^+	5.17 ± 0.07[b] (4)	8.09 ± 0.73[b] (8)
+1.0 L-NNA	3.64 ± 0.29 (4)	6.15 ± 0.15 (8)
+0.1 NMDA	5.85 ± 0.36[b] (4)	5.63 ± 0.20 (4)
+0.01 ODQ	6.42 ± 0.36 [b] (4)	6.62 ± 0.47 (7)
L-NNA 1.0	1.61 ± 0.22 (4)	3.54 ± 0.38 (4)
+50 K^+	3.27 ± 0.21 (4)	4.74 ± 0.67 (4)

The slices were superfused under ischemic conditions (under N_2, no glucose) with the NO-generating compounds from the beginning of the efflux experiments, whereas the other effectors were added at 30 min. The results are rate constants k_2 (32–40 min) $\times 10^{-3}$ min^{-1} with the number of independent experiments in parenthesis. Significance of differences from the corresponding controls: $^{*}P<0.05$; $^{**}P<0.01$ and from the corresponding NO-generating compound: $^{a}P<0.05$; $^{b}P<0.01$; *SNP* sodium nitroprusside; *SNAP* S-nitroso-N-acetylpenicillamine; *HA* hydroxylamine; *L-NNA* N^G-nitro-L-arginine; *NMDA* N-methyl-D-aspartate; *ODQ* 1H-(1,2,4)oxadiazolo(4,3-a)quinoxalin-1-one

In ischemia, SNP, SNAP and HA potentiated basal initial taurine release in developing and adult mice (Table 3, Fig. 2A, B). The SNP effect was further enhanced by K^+, L-NNA, NMDA and ODQ in 7-day-olds, whereas only K^+ was effective in adults (Table 4). Furthermore, the SNAP effect was potentiated by K^+ and NMDA at both ages and by ODQ in the developing mice (Table 4). In adults, the HA effect was increased only by K^+, whereas in developing mice also NMDA and ODQ had such an effect (Table 4).

Discussion

Taurine release in mouse brain stem slices was now shown to be differently modified by NO-generating compounds, both inhibitory and excitatory effects being discernible, as has also occurred in the hippocampus in vitro (Saransaari and Oja, 1999) and in vivo (Watts et al., 2004). In general, NO can modulate neuronal functions via cGMP-dependent and -independent pathways (Prast and Philippu, 2001). The NO-releasing agents used produce NO by different mechanisms. SNP and SNAP form NO extracellularly by spontaneous dissociation (Schuman and Madison, 1994). HA can be regarded as an intracellular generator of NO, since it penetrates easily into cells and is broken down by a catalase-dependent reaction (DeMaster et al., 1989). Its effects might be expected to mimic an increase in intracellular NO production. The varying effects noted with exogenous NO donors may thus also involve the extracellularly produced NO altering the redox state of the NMDA receptor-channel complex (Lei et al., 1992; Lipton et al., 1993) and/or diffusing intracellularly to act on soluble guanylate cyclase (Garthwaite, 1991). Moreover, SNP may also have other effects besides releasing NO, due to its Fe^{2+} and cyanide moieties (Lei et al., 1992). Accordingly, the variable effects of the NO donors used in the present experiments have also been encountered in other cases.

The basal release of taurine under normal conditions was increased by HA, suggesting that NO production evokes taurine release directly without the involvement

of glutamate receptors. SNP and SNAP have also enhanced taurine release from cortical neurons (Chen et al., 1996) and the adult striatum in vivo (Guevara-Guzman et al., 1994). HA has also stimulated the basal release of neurotransmitters, e.g., that of noradrenaline, dopamine, acetylcholine, GABA and glutamate (Lonart et al., 1993), and SNP and SNAP have also potentiated GABA (Ohkuma et al., 1995) and glutamate (Lawrence and Jarrott, 1993; Watts et al., 2005) release. Thus, NO is capable of acting directly upon presynaptic terminals to enhance the release of both transmitters and taurine as a retrograde messenger.

Depolarization by K^+ potentiated the HA-evoked release at both ages and the SNAP-stimulated release in the immature brain stem, indicating that these effects may be additive. NO-generating agents have generally reduced the K^+-evoked release, for example, dopamine release in the bovine retina (Bugnon et al., 1994), glutamate release in cerebellar synaptosomes (Kamisaki et al., 1995) and taurine release in the hippocampus (Saransaari and Oja, 1999). In the presence of high potassium concentrations multiple biosynthetic, exocytotic, storage and/or reuptake processes are likely to occur. On the other hand, depolarization may activate NO synthase, since KCl has been shown to increase cGMP levels in rat cerebellar synaptosomes (Kamisaki et al., 1995).

NO-producing neurons are present in the brain stem (Gotti et al., 2005), as demonstrated for example in the nucleus of the solitary tract and in the ventrolateral medulla (Dun et al., 1994, 1995). Developing brain stem nuclei also express neuronal NO synthase mRNA (Leimert and Blottner, 2003). However, the responses of taurine release were not antagonized by the NO synthase inhibitor L-NNA, which suggests that mechanisms other than NO synthesis might be involved. Only taurine release in the presence of SNP and SNAP in developing mice was affected by ODQ, the inhibitor of soluble guanylate cyclase, indicating likely involvement of cGMP. On the other hand, the SNAP-induced release has been inhibited by ODQ in the adult hippocampus in vivo (Watts et al., 2004) but not in the striatum (Guevara-Guzman et al., 1994).

In contrast to the hippocampus (Saransaari and Oja, 1997a), the ionotrophic glutamate receptor agonist NMDA had no effect in the brain stem of adults, whereas a significant potentiation has been observed in developing mice (Saransaari and Oja, 2006). In keeping with this both the SNAP- and HA-induced releases were further enhanced by NMDA in the immature brain stem, as also the HA-stimulated release in adults, whereas in the hippocampus the NMDA-evoked release of taurine has been reduced by NO donors, particularly by SNP (Saransaari and Oja, 1999). The activation of NMDA receptors induces NO synthesis in the hippocampus (Garthwaite, 1991), whereas the endogenous production of NO is responsible for a sustained blockade of NMDA receptor activity (Manzoni et al., 1992). The NO-generating agents, SNP in particular, diminish NMDA-mediated changes in intracellular Ca^{2+} in neurons, independently of the redox state of NMDA receptors and cGMP production (Hoyt et al., 1992; Tanaka et al., 1993; Omerovic et al., 1994), and block glutamate accumulation (Oh and McCaslin, 1995). The location and timing of NO synthesis are apparently important for the effects of NO to become manifest (Izumi et al., 1992). Moreover, different doses of NO donors can increase or reduce neurotransmitter release, as has been the case with SNP-induced striatal dopamine release (Zhu and Luo, 1992). A biphasic modulation of the release of GABA, glutamate and aspartate by SNAP and nitric oxide synthase inhibitors has also been observed in the hippocampus in vivo (Segieth et al., 1995; Getting et al., 1996). The overall degree of neuronal excitability may depend on the concentration of NO and subsequent increases or decreases in the release of inhibitory (taurine and GABA) and excitatory amino acids. It has even been suggested that complex relationships exist between the releases of these amino acids and that endogenous NO plays an important role in regulating these releases (Watts et al., 2005).

NMDA enhanced the effects of NO donors particularly in the immature hippocampus. NO is produced in immature brain tissue, but the activity of cytosolic NO synthase in the brain is much lower in developing than in adult rats (Keelan et al., 1996). NO synthase activity appears postnatally in the mouse brain, preceding the rapid phase of synaptogenesis consistent with a role of NO in synaptogenesis and synaptic plasticity (Ogilvie et al., 1995; Zhang and Snyder, 1995). NO production seems to be sufficient to enhance the NMDA-evoked taurine release in the immature brain stem, the potentiating effect resulting from the complex redox chemistry of NO actions on the NMDA receptor channel (Lipton et al., 1993). However, the resistance of the immature brain to ischemia/reperfusion might be due to a relative lack of NO production from immature nerve terminals. In any case, the potentiated NMDA-evoked release of taurine by NO donors may be an important mechanism in protecting developing brain tissue against excitotoxicity.

Taurine release is greatly enhanced under ischemic conditions in the brain stem of both adult and developing mice (Saransaari and Oja, 2007). The ischemia-induced

release consisted of both Ca^{2+}-dependent and -independent components and was mediated by Na^+-, Cl^--dependent transporters operating outwards. A part of the release occurred through ion channels and was also modulated by cGMP second messenger systems. All NO donors tested potentiated this ischemia-induced release, as has also been the case in the hippocampus (Saransaari and Oja, 2002). Moreover, K^+ stimulation evoked the release further, resulting in a fairly massive taurine release in the brain stem in this situation. Cerebral ischemia has profound effects on the biosynthetic pathway of NO, affecting both the synthesis of NO and the expression of the genes encoding NOS, the increasing NO production then being accompanied by upregulation of both NOS activity and NOS gene expression (Iadecola, 1997; Matsui et al., 1999). In ischemia, the extracellular levels of cGMP have been increased (Globus et al., 1995; Fedele and Raiteri, 1999), showing that the NO/cGMP pathway also operates in this situation. In adults, neither L-NNA nor ODQ had an effect on the NO-stimulated taurine release in ischemia, indicating that NO synthase may not be involved. On the other hand, the stimulation of this release by ODQ in developing mice seems peculiar, probably resulting from the general effects of ischemia, when neurons are suddenly depolarized, this being accompanied by an increase in extracellular K^+ and a decrease in extracellular Na^+ (Somjen et al., 1990). ODQ might also have other unspecific effects in immature brain tissue, even though it is regarded a rather specific inhibitor of the NO-cGMP pathway. ODQ does not interfere with any of the steps leading to NO synthesis, does not inhibit NO synthase activity and does not inactivate NO (Garthwaite et al., 1995). In adults, NMDA potentiated only the SNAP-induced taurine release in ischemia, while in developing mice this ionotropic glutamate agonist was highly effective. NMDA generally greatly enhances taurine release, in immature brain tissue in particular (Saransaari and Oja, 1997a, 2003), and also under ischemic conditions (Saransaari and Oja, 1997b). It seems that the protective effect of enhanced taurine release by NO production together with NMDA receptor activation is very important particularly in the immature brain stem, which is vulnerable to ischemic neuronal damage.

In conclusion, the effects of NO on taurine release in the brain stem were rather complex and difficult to explain, as might be expected considering the multifunctional role of NO in the central nervous system. The basal initial release and the K^+-stimulated release under normal conditions were enhanced by the NO donors HA, SNAP and SNP. The effects were not antagonized by the NO synthase inhibitor L-NNA, suggesting that mechanisms other than NO synthesis are involved. The SNP-reduced taurine release in developing mice was only affected by the inhibitor of soluble guanylate cyclase, ODQ, indicating possible involvement of cGMP. Also in the immature brain stem NMDA further increased the NO-evoked taurine release. Under ischemic conditions both K^+ stimulation and NMDA potentiated the NO-induced release, particularly in developing mice, probably without the involvement of NO synthase or cGMP. The substantial release of taurine in the developing brain stem evoked by NO donors together with NMDA could constitute an important mechanism against excitotoxicity, protecting the brain stem under cell-damaging conditions.

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