Amino Acids (2000) 19: 547–559

Amino
Acids

Sodium nitroprusside-induced seizure and taurine release from rat hippocampus

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Accepted January 31, 2000

Summary. We have recently reported that the nitric oxide (NO) donor, sodium nitroprusside (SNP), induces seizures which are associated with an increase in the basal release of aspartate and glutamate from rat hippocampus (Kaku et al., 1998). In order to determine whether taurine release occurs with SNP-induced seizures, we examined the effects of NO-related compounds, i.e., the NO trapper, diethyldithiocarbamate (DETC), the superoxide radical scavenger, dithiothreitol (DTT), the xanthine oxidase inhibitor, oxypurinol and the guanylyl cyclase inhibitor, 1H-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one (ODQ), on SNP-induced seizures and *in vivo* taurine release from rat hippocampus using microdialysis. Perfusion with 0.5 mM SNP provoked seizures and significantly increased taurine release, with the increase in release occurring primarily during reperfusion with artificial cerebrospinal fluid lacking SNP. Perfusion with 5 mM DETC significantly abolished the SNP-induced seizures and reduced taurine release during and after perfusion with the drugs. Perfusion with 1 mM DTT significantly reduced both the frequency of the SNP-induced seizures and taurine release during and after perfusion with the drugs. Perfusion with 1 mM oxypurinol or 0.5 mM ODQ did not reduce the frequency of the SNP-induced seizures, but tended to decrease taurine release during and after perfusion with the drugs. These results demonstrate that SNP-induced seizures are triggered by an increase in both NO and peroxynitrite and are related to an increase in taurine release from rat hippocampus.

Keywords: Amino acids – Seizure – Taurine – Nitric oxide (NO) – Sodium nitroprusside – Hippocampus – Microdialysis

Introduction

Nitric oxide (NO) is a molecular mediator that has been implicated in many physiological and pathological processes (Bredt and Snyder, 1994; Szabo,

1996). It remains controversial whether NO acts as a pro- or anticonvulsant (for example, Alexander et al., 1998; Penix et al., 1994).

De Sarro et al. (1993) observed that microinjection of an NO donor, sodium nitroprusside (SNP), into the rat deep prepiriform cortex induced seizures which were prevented by methylene blue, a soluble guanylate cyclase inhibitor. Bagetta et al. (1993) also reported that intrahippocampal microinjection of SNP produced epileptogenic discharges in freely moving rats. In contrast, Marangoz et al. (1994) showed that intracortical microinjection of SNP decreased epileptiform discharges elicited by penicillin. Guevara-Guzman et al. (1994) reported that NO donors and cyclic GMP agonists increased extracellular concentrations of aspartate, glutamate, GABA and taurine in rat striatum. Saransaari and Oja (1999) have recently shown that NO donors modify basal K^+- and NMDA-evoked release of taurine in mouse hippocampal slices. We have recently reported that SNP induces seizures which are associated with an increase in the release of aspartate and glutamate from rat hippocampus (Kaku et al., 1998).

However, to the best of our knowledge, there are no *in vivo* studies regarding the involvement of NO on seizures and taurine release. Therefore, to study further the role of NO in SNP-induced seizures, we examined the effects of various NO-related compounds [diethyldithiocarbamate (DETC, an NO trapper), dithiothreitol (DTT, a superoxide radical scavenger), oxypurinol (a xanthine oxidase inhibitor), and 1H-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one (ODQ, a specific guanylyl cyclase inhibitor)] on SNP-induced seizures and *in vivo* taurine release from rat hippocampus as detected by microdialysis.

We report here that increases in both NO and peroxynitrite mediated by SNP induce seizures, a result related to an increase in taurine release from rat hippocampus.

Materials and methods

The experimental procedures have been described in detail in our previous report (Hada et al., 1998). The Guiding Principles for Care and Use of Animals in the Field of Physiological Sciences (The Physiological Society of Japan) was strictly followed. In brief, male Wister rats weighing 280–320 g were anesthetized with urethane (1.2 g/kg, i.p.). A bipolar stimulating electrode and a recording electrode were inserted into the dorsal hippocampus. The extracellular DC potentials and population spikes were recorded from the CA1 pyramidal cell layer with a glass-microelectrode (placed nearby a microdialysis probe). We stereotaxically implanted a microdialysis probe with 2 mm active membrane (CMA/10, CMA/Microdialysis) into the dorsal hippocampus to apply drugs and to collect dialysates. The basal perfusion medium was an artificial cerebrospinal fluid (ACSF) (composition in mM: NaCl 132.8; KCl 3.0; $CaCl_2$ 2.0; $MgCl_2$ 0.7; $NaHCO_3$ 24.6; urea 6.7; glucose 3.7). The perfusion flow rate was 2.0 μl/min and controlled by a microinjection pump (CMA/100, CMA/Microdialysis).

Experimental protocol

Nine groups of rats were dialysed through probes with 2 mm active membrane length. Five rats served as control and received 100 mM K^+ alone for 30 min (control group). Five

rats received 0.5 mM SNP alone for 30 min (SNP alone group). Six rats received 0.05 mM SNP + 100 mM K^+ for 30 min (0.05 mM SNP group). Five rats received 0.5 mM SNP + 100 mM K^+ for 30 min (0.5 mM SNP group). Six rats received 5 mM SNP + 100 mM K^+ for 30 min (5 mM SNP group). Five rats received 0.5 mM SNP + 5 mM DETC + 100 mM K^+ for 30 min (DETC group). Six rats received 0.5 mM SNP + 1 mM DTT + 100 mM K^+ for 30 min (DTT group). Five rats received 0.5 mM SNP + 1 mM oxypurinol (OP) + 100 mM K^+ for 30 min (oxypurinol group). Six rats received 0.5 mM SNP + 0.5 mM ODQ + 100 mM K^+ for 30 min (ODQ group). SNP, DETC, DTT, oxypurinol or ODQ was applied 30 min before perfusion with 100 mM K^+ for 30 min and thus the total perfusion time was 60 min. Dialysates were collected every 10 min.

Amino acid analysis

Concentrations of aspartate, glutamate and taurine were determined after precolumn derivatization with o-phthaldialdehyde by high performance liquid chromatography using a fluorescence detector (CMA/280, CMA/Microdialysis). A capillary column (BAS, C-18, 5 μm, monomeric 1.0 ø $\times$ 100 mm) was used for amino acid analysis. The mobile phase was phosphate buffer (pH 6.0) with 0.1 mM EDTA-2Na, 10% acetonitrile and 3% tetrahydrofuran and was pumped at a flow rate of 60 μl/min.

Drugs

Sodium nitroprusside (SNP), diethyldithiocarbamate (DETC), dithiothreitol (DTT), oxypurinol (OP), and 1H-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one (ODQ) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in ACSF.

Statistical analysis

The data are expressed as means $\pm$ S.E.M. In order to determine the difference between experimental groups and time course of taurine release, statistical analysis was performed by two way analysis of variance (ANOVA) with repeated measures and post-hoc tests (Fisher's protected least significant difference). For single comparisons, the significance of differences between means was determined by Student's two-tailed t-tests. Total evoked taurine release is the cumulative amount of taurine released during a 30-min period during high K^+ perfusion. P values < 0.05 were considered to be significant.

Results

SNP-induced seizure

Perfusion with 0.5 mM SNP alone induced seizures which were characterized by high frequency, high amplitude spike discharges followed by burst discharges (SNP-induced seizures) (Fig. 1). The seizure discharges appeared with latencies ranging from 70 to 80 min after the start of SNP perfusion and persisted until the end of perfusate collection by microdialysis. The SNP-induced seizures were induced in all 5 rats tested ($P < 0.005$, Table 1). Perfusion with 100 mM K^+ alone never induced seizures during reperfusion with ACSF. Co-perfusion of 100 mM K^+ with 0.5 and 5 mM SNP, but not with 0.05 mM SNP, always induced seizures ($P < 0.005$ and $P < 0.001$, respectively) (Table 1).

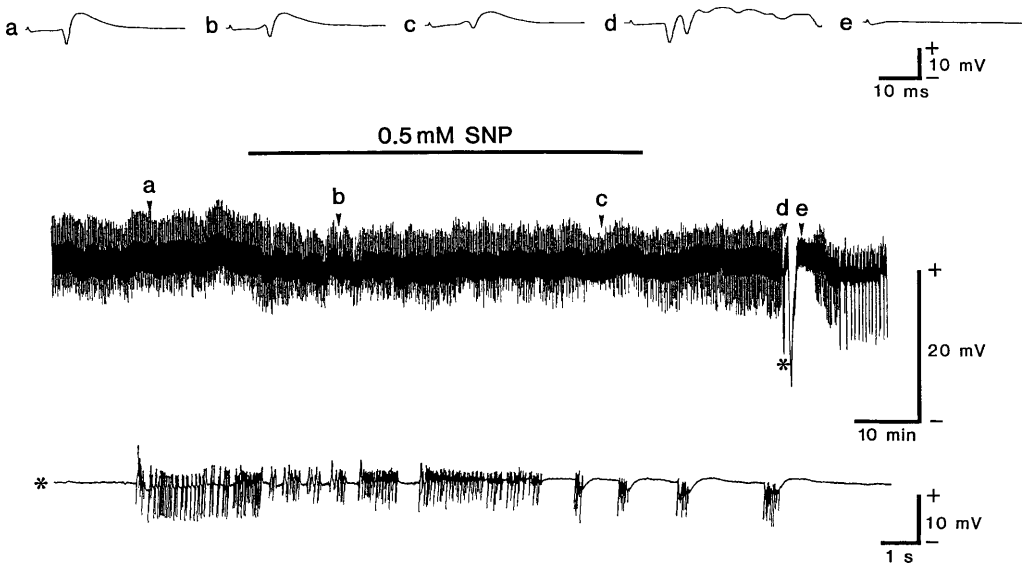

Fig. 1. Typical examples of DC potential activity and EEG activity before, during and after exposure to 0.5 mM SNP alone (middle trace). Population spikes are superimposed on DC potential records. Population spikes recorded at the time points marked by lettered arrow heads are displayed at a faster sweep (upper traces). At the time point marked by an asterisk, seizure spikes occurred and were displayed at a faster sweep in the lower trace. Note that perfusion with 0.5 mM SNP alone always induced seizures which were characterized by high frequency, high amplitude spike discharges followed by burst discharges

Table 1. Effects of various agents on SNP-induced seizures. Each number indicates the number of rats tested. $^{a}P < 0.01$, $^{b}P < 0.005$, $^{c}P < 0.001$, compared with 100 mM K^+ alone (χ^2-test). $^{d}P < 0.025$, $^{e}P < 0.005$, compared with 0.5 mM SNP + 100 mM K^+

Agents	No seizure	Seizure	Total
100 mM K^+ alone	5	0	5
0.5 mM SNP alone	0	5[b]	5
0.05 mM SNP + 100 mM K^+	6	0	6
0.5 mM SNP + 100 mM K^+	0	5[b]	5
5 mM SNP + 100 mM K^+	0	6[c]	6
0.5 mM SNP + 5 mM DETC + 100 mM K^+	5	0[e]	5
0.5 mM SNP + 1 mM DTT + 100 mM K^+	4	2[d]	6
0.5 mM SNP + 1 mM OP + 100 mM K^+	0	5[b]	5
0.5 mM SNP + 0.5 mM ODQ + 100 mM K^+	1	5[a]	6

Effects of NO-related compounds on SNP-induced seizure

To assess whether the seizures were due to the generation of NO, we examined whether SNP induced seizures in the presence of DETC, the NO trapper. Perfusion with 5 mM DETC blocked the SNP-induced seizures in all 5 rats tested ($P < 0.005$, Table 1). NO is known to generate peroxynitrite in the presence of oxygen by binding to superoxide ions. Therefore, experiments

examining whether peroxynitrite was responsible for generation of seizures were conducted. In this study, we examined the effects of DTT, the superoxide radical scavenger, on seizures. We reasoned that DTT should attenuate the formation of peroxynitrite and consequently abolish or reduce seizures. Perfusion with 1 mM DTT significantly reduced the frequency of SNP-induced seizures ($P < 0.025$), when compared with 0.5 mM SNP group (Table 1).

We reasoned that xanthine oxidase should be involved in peroxynitrite formation under these experimental conditions. Therefore, we examined the effects of oxypurinol (OP), the xanthine oxidase inhibitor, on seizures. However, perfusion with 1 mM oxypurinol failed to prevent seizures in all 5 rats tested.

To test whether seizures were associated with activation of guanylate cyclase, we examined whether SNP induced seizures in the presence of ODQ, the guanylyl cyclase inhibitor. Perfusion with 0.5 mM ODQ failed to prevent SNP-induced seizures in 5 of 6 rats tested.

Effects of NO-related compounds on taurine release

The basal levels of key amino acids were as follows: $0.149 \pm 0.009\,\mu M$ ($n = 15$) for aspartate; $0.469 \pm 0.051\,\mu M$ ($n = 15$) for glutamate; $1.911 \pm 0.210\,\mu M$ ($n = 15$) for taurine. Figure 2 shows the time course for changes in taurine release from the hippocampus before, during and after perfusion with 0.5 mM SNP alone. While SNP perfusion for 60 min did not affect taurine release during the 60-min SNP perfusion, it increased taurine release 90 min after the start of SNP perfusion by 30%.

Figures 3A and 4A show the time course for high K^+-evoked taurine release in high K^+ alone (control), 0.05, 0.5, 5 mM SNP, DETC, DTT, oxypurinol (OP) and ODQ. In all groups, perfusion with 100 mM K^+ increased taurine release in a similar pattern. Perfusion with high K^+ alone increased taurine release to a maximum value of $701.6 \pm 74.8\%$ ($n = 5$) (Fig. 3A). Perfusion with 0.05, 0.5 and 5 mM SNP increased the high K^+-evoked taurine release to maximum values of $594.8 \pm 73.3\%$ ($n = 6$), $1143.4 \pm 144.6\%$ ($n = 5$) and $589.7 \pm 100.8\%$ ($n = 6$), respectively. Perfusion with 5 mM DETC, 1 mM DTT, 1 mM oxypurinol (OP) and 0.5 mM ODQ in the presence of 0.5 mM SNP significantly increased the high K^+-evoked taurine release to maximum values of $396.6 \pm 43.2\%$ ($n = 5$), $558.5 \pm 63.5\%$ ($n = 6$), $859.0 \pm 101.3\%$ ($n = 5$) and $1027.4 \pm 376.1\%$ ($n = 3$), respectively, 70 min after the onset of SNP perfusion (Fig. 4A).

To better assess the effects of these agents, we estimated the total amount of taurine released during the 30-min high K^+ perfusion in each group. When compared with the control group ($100.0 \pm 12.2\%$; $n = 5$), the 5 mM SNP group significantly decreased taurine release to $48.9 \pm 8.9\%$ ($n = 6$; $P < 0.02$), but the 0.05 and 0.5 mM SNP groups did not cause any appreciable change: $78.9 \pm 10.0\%$ ($n = 6$) for 0.05 mM SNP; $121.2 \pm 19.3\%$ ($n = 5$) for 0.5 mM SNP (Fig. 3B). When compared with the 0.5 mM SNP group ($100.0 \pm 16.0\%$; $n = 5$),

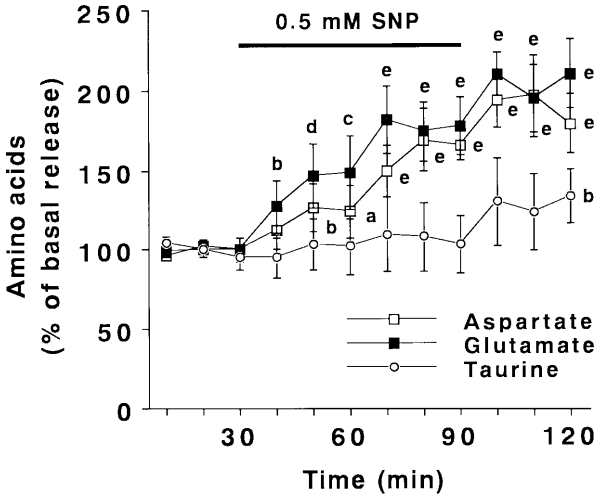

Fig. 2. Time course of the release of aspartate, glutamate and taurine from hippocampus before, during and after perfusion of 0.5 mM SNP alone. Each point with a vertical bar represents the mean ± SEM of amino acids released for 5 experiments. The aspartate and glutamate release began to increase 10 min after the onset of SNP perfusion. In contrast, the basal taurine level did not change during the 60-min SNP perfusion and increased 90 min after the start of SNP perfusion by 30%. The relative amounts of SNP-induced taurine release were at any time point lower than those of aspartate and glutamate. Significant differences in amounts of release of aspartate, glutamate and taurine during and after SNP perfusion as compared with the mean baseline values are marked by *a* ($P < 0.05$), *b* ($P < 0.02$), *c* ($P < 0.01$), *d* ($P < 0.005$) and *e* ($P < 0.001$), respectively

perfusion of 5 mM DETC and 1 mM DTT with 0.5 mM SNP significantly reduced taurine release to 38.1 ± 6.6% ($n = 5$; $P < 0.02$) and 38.7 ± 4.8% ($n = 6$; $P < 0.01$), respectively (Fig. 4B). However, perfusion of 1 mM oxypurinol (OP) and 0.5 mM ODQ with 0.5 mM SNP did not cause any appreciable change: 67.3 ± 11.5% ($n = 5$) and 115.0 ± 54.7% ($n = 3$), respectively.

Next, we estimated the total amount of taurine released within 30 min after the end of high K^+ perfusion in each group. When compared with the control group (100.0 ± 8.9%; $n = 5$), the 0.5 mM SNP group significantly increased taurine release (316.5 ± 46.3%; $n = 5$) ($P < 0.005$), but the 0.05 and 5 mM SNP groups did not cause any appreciable change: 103.9 ± 17.8% ($n = 6$) and 164.1 ± 37.4% ($n = 6$), respectively (Fig. 3C). Perfusion of 5 mM DETC and 1 mM DTT with 0.5 mM SNP significantly reduced taurine release to 19.4 ± 2.5% ($n = 5$; $P < 0.002$) and 34.6 ± 6.5% ($n = 6$; $P < 0.005$), respectively, when compared with the 0.5 mM SNP group (100.0 ± 14.6%; $n = 5$) (Fig. 4C). Perfusion of 1 mM oxypurinol (OP) and 0.5 mM ODQ with 0.5 mM SNP tended to reduce taurine release, but not significantly (61.2 ± 6.5%; $n = 5$ and 56.6 ± 18.0%; $n = 3$, respectively).

Discussion

The principal findings in the present *in vivo* study were as follows: (1) the NO donor, SNP at 0.5 mM always induced seizures (SNP-induced seizures) and

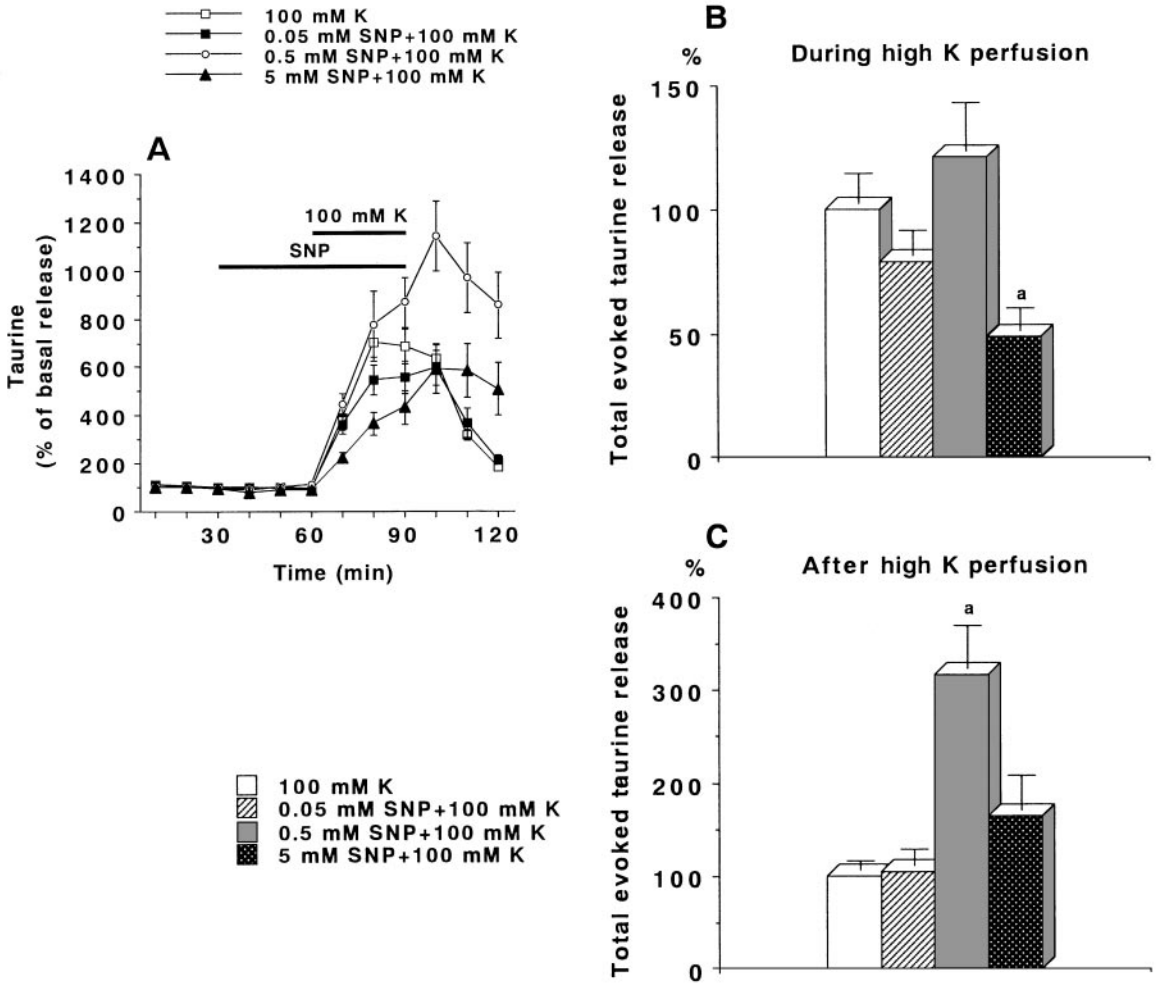

Fig. 3. **A** Time course of the taurine release evoked by perfusion with 100 mM K^+ alone (□; n = 5), 0.05 mM SNP + 100 mM K^+ (■; n = 6), 0.5 mM SNP + 100 mM K^+ (○; n = 5) or 5 mM SNP + 100 mM K^+ (▲; n = 6). Each point with a vertical bar represents the mean ± SEM of taurine released for 5–6 experiments. An ANOVA computed on high K^+-evoked taurine release revealed that main effects of group and time, and an interaction between group and time were significant ($F(3/18) = 7.147$, $P = 0.0023$; $F(11/198) = 108.219$, $P < 0.0001$; $F(33/198) = 8.282$, $P < 0.0001$, respectively). Fisher's *post-hoc* tests revealed significant differences between the high K^+ and the 0.5 mM SNP groups, between the 0.05 and the 0.5 mM SNP groups and between the 0.5 and the 5 mM SNP groups ($P = 0.0032$, $P = 0.0008$ and $P = 0.0009$, respectively). **B** The effect of SNP on the total amount of taurine release during high K^+ perfusion. The total amount of taurine release was obtained by summing up the amount of taurine released during the 30-min high K^+ perfusion. Each column with a vertical bar represents the mean ± SEM obtained from 5–6 animals. Note that the 5 mM SNP group significantly reduced the taurine release, when compared with the high K^+ alone group ($P < 0.02$). **C** The effect of SNP on the total amount of the taurine release after high K^+ perfusion. The total amount of taurine release was obtained by summing up the amount of taurine released for 30 min after the end of high K^+ perfusion. Each column with a vertical bar represents the mean ± SEM obtained from 5–6 animals. Note that the 0.5 mM SNP group significantly increased the taurine release after high K^+ perfusion, when compared with the high K^+ alone group ($P < 0.005$)

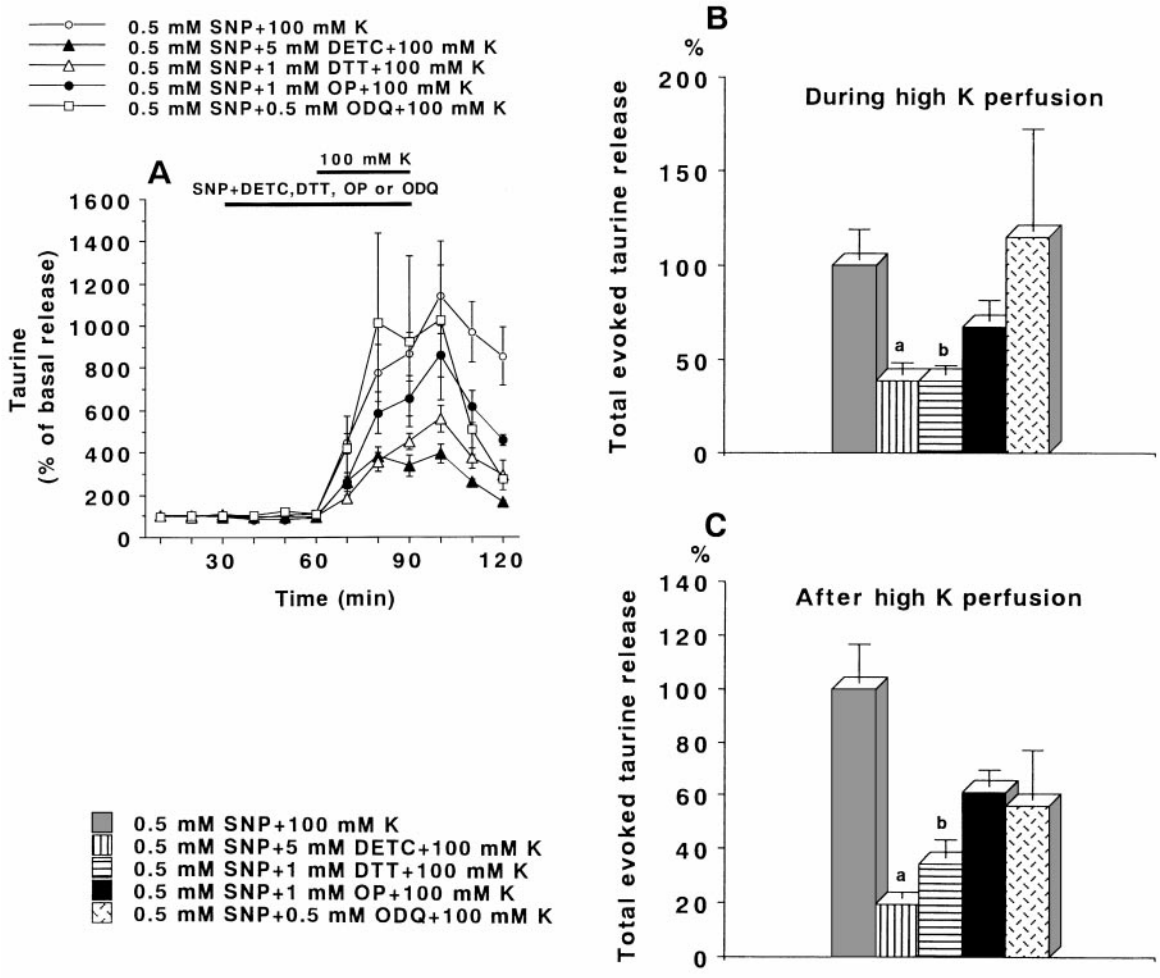

Fig. 4. **A** Time course of the taurine release evoked by perfusion with 0.5 mM SNP + 100 mM K^+ (○; n = 5), 0.5 mM SNP + 5 mM DETC + 100 mM K^+ (▲; n = 5), 0.5 mM SNP + 1 mM DTT + 100 mM K^+ (△; n = 6), 0.5 mM SNP + 1 mM oxypurinol (OP) + 100 mM K^+ (●; n = 5) or 0.5 mM SNP + 0.5 mM ODQ + 100 mM K^+ (□; n = 3). Each point with a vertical bar represents the mean ± SEM of taurine released for 3–6 experiments. An ANOVA computed on high K^+-evoked taurine release revealed that main effects of group and time, and an interaction between group and time were significant ($F(4/19) = 6{,}638$, $P = 0.0016$; $F(11/209) = 78.383$, $P < 0.0001$; $F(44/209) = 5.164$, $P < 0.0001$, respectively). Fisher's *post-hoc* tests revealed significant differences between the 0.5 mM SNP and the DETC groups, between the 0.5 mM SNP and the DTT groups and between the 0.5 mM SNP and the oxypurinol (OP) groups ($P = 0.0003$, $P = 0.0007$ and $P = 0.0367$, respectively). **B** The effects of DETC, DTT, oxypurinol (OP) and ODQ on the total amount of taurine release in the presence of SNP during high K^+ perfusion. The total amount of taurine release was obtained by summing up the amount of taurine released during the 30-min high K^+ perfusion. Each column with a vertical bar represents the mean ± SEM obtained from 3–6 animals. Note that perfusion with 5 mM DETC and 1 mM DTT in the presence of 0.5 mM SNP significantly reduced the taurine release during high K^+ perfusion ($P < 0.02$ and $P < 0.01$, respectively), when compared with the 0.5 mM SNP group. **C** The effects of DETC, DTT, oxypurinol (OP) and ODQ on the total amount of the taurine release in the presence of SNP after high K^+ perfusion. The total amount of taurine release was obtained by summing up the amount of taurine released for 30 min afte the end of high K^+ perfusion. Each column with a vertical bar represents the mean ± SEM obtained from 3–6 animals. Note that perfusion with 5 mM DETC and 1 mM DTT in the presence of 0.5 mM SNP significantly enhanced the taurine release after high K^+ perfusion ($P < 0.002$ and $P < 0.005$, respectively), when compared with the 0.5 mM SNP group

increased taurine release from rat hippocampus after high K^+ perfusion; (2) the NO trapper, DETC abolished SNP-induced seizures and the superoxide radical scavenger, DTT reduced the frequency of the SNP-induced seizures. Both DETC and DTT reduced taurine release during and after perfusion with high K^+; (3) both the xanthine oxidase inhibitor, oxypurinol and the specific guanylyl cyclase inhibitor, ODQ, did not affect the SNP-induced seizures but tended to reduce taurine release after high K^+ perfusion.

Mechanisms of genesis of the SNP-induced seizures

The present result that SNP induces seizures is supported by the previous findings by De Sarro et al. (1993) and Bagetta et al. (1993). De Sarro et al. (1993) observed that microinjection of SNP into the rat deep prepiriform cortex induced seizures which were prevented by methylene blue, a soluble guanylate cyclase inhibitor. Bagetta et al. (1993) also reported that intrahippocampal microinjections of SNP produced epileptogenic discharges in freely moving rats. In contrast, Marangoz et al. (1994) reported that intracortical microinjections of SNP decreased epileptiform discharges elicited by penicillin. Differences in concentrations of SNP, seizure models and brain regions might explain the apparent contradiction.

Relationship between SNP-induced seizure and glutamate release

In the present study, the increase in glutamate release began approximately 70–80 min after SNP perfusion was started, and sustained until the end of the experiment (Fig. 2). This increase in extracellular glutamate concentration corresponded with the appearance of SNP-induced seizures. Our findings are in accordance with a view that glutamatergic neurotransmission is involved in the initiation, propagation and maintenance of seizures (Rogawski, 1995; Urbanska et al., 1998 for reviews). For example, the extracellular glutamate concentration increased with seizures induced by the cholinesterase inhibitor, soman (Wade et al., 1987; Lallement et al., 1991) and in epileptic patients (Carson et al., 1992; During and Spencer, 1993). In pilocarpine seizure models, marked increases in extracellular hippocampal glutamate levels appeared (Millan et al., 1993). Moreover, SNP inhibited ^{3}H-glutamate uptake in rat hippocampal synaptosomes (Pogun et al., 1994), indicating that the inhibition of glutamate uptake by NO could play a role in the toxicity of glutamate. Indeed, NO induced Ca^{2+}-independent glutamate release from rat synaptosomes (McNaught and Brown, 1998). Hammarstrom and Gage (1999) have recently observed in rat hippocampal neurons that SNP increases persistent sodium current. Thus, SNP may markedly increase the excitability of hippocampal neurons and induce the generation of seizures.

Peroxynitrite produced by both NO and superoxide ions may, in part, be responsible for the generation of the seizures. This view is supported by our previous (Kaku et al., 1998) and present results. The NO trapper, DETC, would catch excessive exogenous NO produced by SNP, reduce aspartate and

glutamate release, and consequently abolish the SNP-induced seizures. The SNP-induced seizures were partially blocked by application of DTT, the superoxide radical scavenger, which prevented peroxynitrite formation. Therefore, it may be reasonable to suggest that peroxynitrite may partially contribute to the genesis of the SNP-induced seizures. Inhibition of glutamate uptake by peroxynitrite might contribute to the build up of excitotoxic extracellular glutamate (Trotti et al., 1996), resulting in the seizure generation. However, in the present study DTT did not completely abolish the SNP-induced seizures. The reason may be explained by the enhancement of the NMDA response (Aizenman et al., 1989) and NMDA-induced Ca^{2+} influx by perfusion of DTT (Reynolds et al., 1990). Indeed, Tolliver and Pellmar (1987) have observed that DTT elicits epileptiform activity in the CA1 pyramidal neurons of the guinea pig hippocampal slice. It is probable that the SNP-induced seizures may be caused by effects of Fe^{2+} and cyanide moieties. However, this probability can be ruled out, because DETC completely abolished the SNP-induced seizures. Therefore, we think that the SNP-induced seizures may be caused by both NO and peroxynitrite.

The xanthine oxidase inhibitor (allopurinol) has been reported to exert antiepileptic effects in epileptic patients (De-Marco et al., 1988; Tada et al., 1991) and in the epileptic mutant EL mouse (Murashima et al., 1996; 1998). It is unclear why oxypurinol (an allopurinol matabolite) failed to prevent the SNP-induced seizures in this study. However, we speculate that because superoxide radicals produced by xanthine oxidase may not contribute to the generation of the SNP-induced seizures, consequently oxypurinol might fail to prevent the SNP-induced seizures. In the present experimental conditions, basal and high K^+-evoked release of hypoxanthine might be so low that only a small amount of O_2^- (superoxide anion) could be produced. Consequently, oxypurinol would not have affected the SNP-induced seizures. This notion is supported by the finding of O'Regan et al. (1997) showing that application of xanthine oxidase with xanthine significantly enhances glutamate accumulation during reperfusion in rat cortex, as compared with application of xanthine oxidase alone.

In this study, ODQ, the inhibitor of guanylyl cyclase, did not affect the SNP-induced seizures. In contrast, De Sarro et al. (1993) showed that an analogue of cyclic GMP, 8-bromo-cyclic GMP, induced seizures which were prevented by methylene blue, another inhibitor of guanylyl cyclase. At present we have no reasonable explanation for the observation that ODQ did not abolish the SNP-induced seizures. It might be due to differences between actions of methylene blue and ODQ, which are unrelated to cyclic GMP. Indeed, Mayer et al. (1993) suggested that methylene blue acted as a direct inhibitor of NO synthase.

Mechanisms of SNP-induced increase in taurine release

In this study, the SNP-induced seizures were closely associated with increases in taurine release from rat hippocampus (Figs. 1 and 2). Moreover, both the

NO trapper, DETC, and the superoxide radical scavenger, DTT, reduced the SNP-induced increase in taurine release. These results may indicate that the increase in taurine release induced by SNP are mediated by both NO and peroxynitrite. These findings are supported by other reports. Chen et al. (1996) have observed that NO donors increase [^{3}H]taurine release from mouse cerebral cortical neurons in primary culture. Moreover, Saransaari and Oja (1999) have recently shown that NO donors, such as SNP and hydroxylamine, increase taurine release in mouse hippocampal slices.

The increase in aspartate and glutamate release should trigger the influx of Na^+ and Ca^{2+} into postsynaptic cells. Massive influx of these ions should cause water to follow, leading to cellular swelling. The seizures induced by SNP would also evoke cellular swelling which in turn leads to taurine release and regulates osmotic imbalance. Moreover, the released taurine would act on presynaptic terminals to inhibit glutamate release (Kamisaki et al., 1993), hyperpolarize postsynaptic neurons (Taber et al., 1986; Zeize, 1986) and thus reduce neuronal hyperexcitation under pathological conditions such as seizures. Previous studies have shown that taurine has neuroprotective activity against epilepsy (Durelli and Mutani, 1983; Oja and Kontro, 1983; Toth et al., 1983). El Idrissi and Trenkner (1999) have recently shown in cultures of mouse cerebellar granule cells that taurine, as well as basic fibroblast growth factor, prevents glutamate excitotoxicity through the regulation of $[Ca^{2+}]_i$ and mitochondrial energy metabolism.

Inhibition of guanylyl cyclase with ODQ tended to reduce SNP-induced taurine release, although the effect was not significant. These findings are not in agreement with the findings of Guevara-Guzman et al. (1994) showing that cyclic GMP agonists increase taurine release from rat striatum using microdialysis. The SNP-induced increase in taurine release in the present study may be mediated partly through a cyclic GMP-independent mechanism.

In conclusion, these results demonstrate that SNP-induced seizures are triggered by an increase in both NO and peroxynitrite and related to an increase in taurine release from rat hippocampus.

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Received January 25, 2000