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Excessive release of [^{3}H]noradrenaline and glutamate in response to simulation of ischemic conditions in rat spinal cord slice preparation: effect of NMDA and AMPA receptor antagonists

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Abstract

In the present study we investigated the effects of NMDA and non-NMDA glutamate receptor antagonists on the ischemia-evoked release of [^{3}H]noradrenaline from rat spinal cord slices. An in vitro ischemia model (oxygen and glucose deprivation) was used to simulate the ischemic conditions known to cause neuronal injury. Spinal cord slices were loaded with [^{3}H]noradrenaline and superfused with Krebs solution in a micro-organ bath. Both axonal stimulation and ischemia increased the release of [^{3}H]noradrenaline, but the release in response to glucose and oxygen deprivation was $[Ca^{2+}]_o$ independent. Dizocilpine (MK-801), an NMDA receptor antagonist, suppressed the release of [^{3}H]noradrenaline produced by ischemia, while it enhanced the release of [^{3}H]noradrenaline evoked by electrical field stimulation. In contrast, LY300168 (GYKI-53655) [(±)-3-*N*-methylcarbamyde-1-(4-aminophenyl)-4-methyl-1.8-methylene-dioxy-5*H*-2.3-benzodiazepine] and its (−)isomer LY303070 (GYKI-53784) [(−)-3-*N*-methylcarbamyde-1-(4-aminophenyl)-4-methyl-1.8-methylene-dioxy-5*H*-2.3-benzodiazepine] AMPA receptor antagonists, had no effect on the release of [^{3}H]noradrenaline evoked by either electrical stimulation or ischemia. Desipramine, a noradrenaline uptake inhibitor, potentiated the release of [^{3}H]noradrenaline evoked by ischemia, while in the absence of $[Ca^{2+}]_o$ but under conditions when [^{3}H]noradrenaline release was further increased, it reduced the release. Dizocilpine also decreased glutamate and aspartate release, measured by high performance liquid chromatography, during ischemia. It is concluded that glutamate release and NMDA receptors, but not AMPA receptors, are involved in the acute effect of oxygen and glucose deprivation on the excessive release of noradrenaline and that this release is not related to physiological axonal conduction.

Keywords: Spinal cord; Ischemia; Noradrenaline release; Carrier-mediated release; NMDA receptor; Non-NMDA receptor; Dizocilpine; LY300168 (GYKI-53655); LY303070 (GYKI-53784)

1. Introduction

Spinal cord and head injuries are complex and multifactorial processes that may involve primary and secondary insults. Hypoxia and subsequent hypoglycemia are the most frequent causes of secondary damage in spinal cord-injured patients and contribute significantly to morbidity and mortality. Glutamate, an excitatory amino acid, is the main fast neurotransmitter in the central nervous system of vertebrates. It has been shown that glutamate, which is known to be neurotoxic, contributes to ischemia-induced neuronal injury (Siesjö, 1988; Koh and Choi, 1991; Kimura et al., 1998).

A growing body of evidence suggests that the excessive release of excitatory amino acids combined with acute insults, including ischemia, hypoglycemia, and trauma, contributes to the secondary damage of neuronal tissue (Wieloch et al., 1985; Lobner and Lipton, 1990; Wrathall et al., 1992; Ross, 1996; Doolette, 1997; Figiel and Kaczmarek, 1997; Matsuoka et al., 1997). Moreover, glutamate receptor antagonists of the NMDA and the non-NMDA subtypes can reduce neurotoxicity following focal ischemia (Faden et al., 1989; Wrathall et al., 1994; Newell et al., 1995; cf. Vizi et al., 1996, 1997). It has been shown that the excessive release of dopamine and noradrenaline

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during ischemia has an injurious effect in the striatum and hippocampus, respectively (Globus et al., 1988, 1989).

During ischemia there is an excessive $[Ca^{2+}]_o$ independent noradrenaline release in the spinal cord (Uchihashi et al., 1998), and there is a strong possibility that noradrenaline released in excessive amounts has an injurious effect (Halonen et al., 1995), potentiating NMDA receptor-mediated injuries in the spinal cord (Wohlberg et al., 1987). While the exocytotic vesicular release of neurotransmitters associated with axonal activity requires energy and is $[Ca^{2+}]_o$ dependent, the resting, non-vesicular release of noradrenaline during ischemia is $[Ca^{2+}]_o$ independent and very sensitive to the intracellular ATP level (Milusheva et al., 1996; Uchihashi et al., 1998; Vizi, 1998). It was suggested, therefore, that noradrenaline release during ischemia caused by reversal of the carrier system does not require energy. This is consistent with the observed decrease in intracellular ATP levels and the subsequent inhibition of sodium pump activity, which leads to a decline in the Na^+ electrochemical gradient across the plasma membrane and to the accumulation of $[Na^+]_i$, resulting in the release of cytoplasmic transmitter (Vizi, 1972, 1978; Milusheva et al., 1996; Vizi and Kiss, 1998).

In the present study we investigated the effect of dizocilpine, an NMDA receptor antagonist, LY300168 (GYKI-53655) and its (−)isomer LY303070 (GYKI-53784), AMPA receptor antagonists, on the release of noradrenaline associated with axonal conduction and evoked by ischemic conditions. Since the release of both glutamate and noradrenaline associated with axonal conduction can be mutually modulated via presynaptic hetero NMDA receptors and auto- and hetero-α_2-adrenoceptors (Kamisaki et al., 1993; Klarica et al., 1996; Umeda et al., 1997), under ischemic conditions the possible presynaptic modulation of glutamate release via α_2-adrenoceptors by noradrenaline was also studied.

2. Materials and methods

2.1. Materials

(−)-Desipramine HCl, and (−)-nicotine hydrogen tartrate were purchased from Sigma (Budapest), and [^{3}H]noradrenaline (10 mCi/ml, 1.81 TB Bq/mmol) was from Amersham Int. (Bucks, UK). Dizocilpine, LY300168 (GYKI-53655) [(±)-3-*N*-methylcarbamyde-1-(4-aminophenyl)-4-methyl-1.8-methylene-dioxy-5*H*-2.3-benzodiazepine] and its (−)isomer LY303070 (GYK-53784) were received from Drug Research Institute (Budapest, Hungary).

2.2. Preparation of spinal cord slices

All experiments were carried out with the permission of the local Animal Care Committee. Male Wistar rats (180–250 g) were lightly anesthetized with diethyl ether and decapitated. The thoracolumbar part of the spinal cord was removed (Ko et al., 1997) and immediately placed into ice-cold and continuously bubbled (95% O_2 and 5% CO_2) Krebs solution (in mmol/l: NaCl, 113; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 2.5; and glucose, 11.5 ascorbic acid, 0.3; N_2EDTA, 0.03). The spinal cord was cleaned of attached tissue and sliced into 0.4-mm thick transverse sections with a McIlwain tissue slicer.

2.3. Release of [^{3}H]noradrenaline

The slices were loaded with 1-[7,8-^{3}H]noradrenaline ([^{3}H]noradrenaline; 10 mCi/ml, 1.81 TBq/mmol; Amersham International plc, Amersham, Bucks, UK) for 30 min at 37°C in Krebs solution containing [^{3}H]noradrenaline (10 μCi/ml) and continuously bubbled with 95% O_2 and 5% CO_2. After loading, the slices were transferred to a microvolume (0.1 ml) perfusion system (Vizi et al., 1985). The preparations were stabilized for 60 min by superfusion with Krebs solution at 37°C at the rate of 0.5 ml/min and continuously bubbled with 95% O_2 and 5% CO_2.

The effluent was collected in 3-min fractions during the subsequent experimental period. At the end of the experiments the slices were homogenized in 0.5 ml 10% trichloroacetic acid for 30 min. Aliquots (0.5 ml) of superfusate and aliquots (0.1 ml) of the tissue supernatant were added to 2 ml Packard Ultima Gold scintillation cocktail. Released [^{3}H]noradrenaline was counted in a Packard-Canberra TR 1900 liquid scintillation counter. The fractional release (FR) is expressed as a percentage of the total tissue tritium content at the onset of the collection period.

Slices were stimulated (40 V, 3 Hz, 1 ms, 240 shocks) through platinum electrodes, using a Grass S88 stimulator (Astro-Med, West Warwick, RI, USA), at the beginning of the 3rd and 13th fractions (S_1 and S_2). Drugs to be tested were added to the preparations 15 min before S_2 and were kept in the solution thereafter.

Ischemia was simulated by having spinal cord slices in Krebs solution lacking glucose, bubbled with 95% N_2 and 5% CO_2, from the 7th fraction to the end of experiments (39 min). Drugs to be tested were added to the preparations 9 min before the beginning of the ischemic condition and were kept in the solution thereafter. When Ca^{2+}-free solution was used, $CaCl_2$ was omitted from the Krebs solution and 1 mM EGTA was added.

2.4. Amino acid high performance liquid chromatography analysis

The contents of glutamate and aspartate in the perfusion media were measured by means of a Gilson liquid chromatographic System (Gilson Medical Electronics, Middleton, WI USA). Two delivery pumps (Model 305, 306), a programmable autoinjector (Model 231–401) with a 'trap' column (15–25 μm Nucleosil C-18 (20 × 4.0 mm)), and a fluorometer (Model 121) were used. Separation of amino

acids was performed on a 3-μm Nucleosil C-18 column (150 × 4.6 mm). Their concentrations were determined by a two point calibration curve internal standard method. The column was equilibrated with solvent A, which consisted of 11.25% methanol–acetonitrile (3.5:1 v/v) in 0.01 M potassium phosphate buffer (pH 7.2). The mobile phase B consisted of 22.2% acetonitrile in methanol, as described earlier (Mally et al., 1996). The analysis was carried out at a flow rate of 1.5 ml/min.

The perfusion fluid was centrifuged at 3000 g for 15 min at 0–4°C, and the supernatant was kept at −70°C until analysis. The reagent mixture was prepared by dissolving 10 mg *o*-phthal dialdehyde in 250 μl methanol and by adding 50 μl of 2-mercapto ethanol in 4.7 ml of 1 M borate buffer (pH 10.5). Derivatization was performed by mixing 50 μl with 600 μl sample and 50 μl of 2 μM α-amino adipic acid as an internal standard 2 min before the sample was injected onto the column.

After the re-equilibration period, 400 μl volume of the derivativized sample was loaded onto the 'trap' column and was washed with buffer A; then the 'trap' column was inserted in the analysis line. The derivatization and the 'bio trap' were performed automatically (with special autosampler program) before the trap column was inserted into the analysis line (Mally et al., 1996).

2.5. Statistical analysis

Statistical analysis was done by two-way analysis of variance (ANOVA) for repeated measures followed by Dunnett's test for multiple comparisons. A P value less than 0.05 was considered statistically significant. Values in the text and figures are expressed as means ± S.E. of n observations.

3. Results

3.1. Release of [^{3}H]noradrenaline evoked by electric field stimulation

After slices were loaded with [^{3}H]noradrenaline, and perfused for 60 min, the average uptake of radioactivity was $5.86 \pm 0.57 \times 10^6$ Bq/g ($n = 21$), and the average resting release in a 3-min fraction period was 0.76 ± 0.04% of the total radioactivity.

Electrical stimulation (40 V, 1 ms, 3 Hz, 240 shocks) released [^{3}H]noradrenaline from the slice preparation. The stimulation-evoked release was completely blocked by tetrodotoxin administered at a concentration of 1 μM (data not shown). The average stimulated release of radioactivity was $1.07 \pm 0.12 \times 10^4$ Bq/g, i.e., 0.86 ± 0.09% of the total radioactivity ($n = 21$) (Fig. 1). When the stimulation was repeated within 30 min, the FRS_2/FRS_1 ratio was 0.85 ± 0.03, indicating that stimulation-evoked release under control conditions was fairly constant. The release

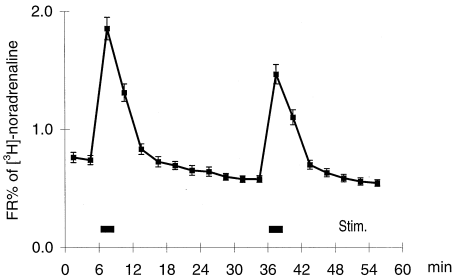

Fig. 1. Release of [^{3}H]noradrenaline from rat spinal cord slices. The slices were stimulated (40 V, 1 ms, 3 Hz, 240 shocks) two times (at the beginning of the 3rd and the 13th fractions). FRS_2/FRS_1 ratio was 0.86 ± 0.02 ($n = 21$). FR, fractional release.

associated with axonal conduction, but not the resting release of radioactivity, was completely $[Ca^{2+}]_o$ dependent: removal of Ca^{2+} and addition of EGTA (1 mM) completely blocked the release of radioactivity associated with electrical stimulation (data not shown).

Since it has been shown (Lodge et al., 1996) that LY300168 (GYKI-53655) after parenteral or oral administration leads to reduced excitation of single neurons in the rat spinal cord by AMPA and also to neuroprotection in the CA1 region of the hippocampus, following bilaterial occlusion of the carotid arteries in the gerbil, we studied the effect of AMPA receptor antagonists.

The release of noradrenaline was studied in the presence of NMDA and non-NMDA (AMPA) receptor antagonists added 15 min before the second stimulation (S_2). The release evoked by the first stimulation (S_1) served as control. While non-NMDA receptor antagonists LY300168 (GYKI-53655) (20 μM) and its (−)isomer LY303070 (GYKI-53784) (20 μM) failed to affect the release, dizocilpine applied at concentrations of 3 and 30 μM enhanced the stimulation-evoked release of [^{3}H]noradrenaline in a concentration-dependent manner (Table 1). In its presence the FRS_2/FRS_1 ratios were significantly higher (1.106 ± 0.05 at 3 μM and 1.650 ± 0.168 at 30 μM) than in control experiments (0.859 ± 0.026) (Table 1). Desipramine (30 μM) also enhanced the stimulation-evoked release of [^{3}H]noradrenaline. When desipramine (30 μM) was added to the superfusion fluid, from 30 min prior to the first fraction and kept in the solution until the end of experiments, thereby excluding any possible effect on the carrier system, dizocilpine added to the superfusion fluid during the second stimulation failed to affect the release of [^{3}H]noradrenaline (control: 0.975 ± 0.138, $n = 4$, in the presence of dizocilpine (30 μM) 1.000 ± 0.053, $n = 4$–4, $P > 0.05$).

3.2. Excessive release of [^{3}H]noradrenaline evoked by ischemia

Ischemia was simulated by subjecting the tissue to hypoxic conditions (95% N_2 + 5% CO_2) and by removing glucose from the medium. Under these conditions, as in

Table 1
Effect of NMDA (dizocilpine) and non-NMDA receptor antagonists (GYKI-53784 and GYKI-53655) on the release of [^{3}H]noradrenaline, associated with axonal conduction

Drugs (μM)	FRS_2/FRS_1	*n*	Significance
1. control	0.859 ± 0.026	21	
2. dizocilpine (3)	1.106 ± 0.050	8	2:1 $P < 0.05$
3. dizocilpine (30)	1.650 ± 0.168	6	3:1 $P < 0.001$
4. LY303070 (GYKI-53784) (20)	0.902 ± 0.041	6	4:1 NS
5. LY303070 (GYKI-53784) (20)	1.005 ± 0.029	4	5:1 NS
+ dizocilpine(3)			5:2 NS
6. desipramine (3)	1.017 ± 0.035	7	6:1 NS
7. desipramine (30)	1.463 ± 0.256	4	7:1 $P < 0.01$
8. LY300168 (GYKI-53655) (20)	0.975 ± 0.018	4	8:1 NS
9. LY300168 (GYKI-53655) (20)	1.090 ± 0.021	4	9:1 NS
+ dizocilpine(3)			9:2 NS
10. LY300168 (GYKI-53655) (20)	1.348 ± 0.095	4	10:1 $P < 0.01$
+ dizocilpine(30)			10:3 NS

Field stimulation was used (3 Hz, 1 ms impulse duration, 240 shocks). Drugs were added between the first (S_1) and second (S_2), stimulation, 15 min prior to the second stimulation. Thirty minutes elapsed between S_1 and S_2. Changes in FRS_2/FRS_1 ratio represents the effect of drugs on [^{3}H]noradrenaline release.
n, Number of experiments.
NS, not significant.
Mean ± S.E.M.

our earlier experiments (Uchihashi et al., 1998), the release of [^{3}H]noradrenaline gradually increased (Fig. 2), reaching a five-fold increase after 40 min. When electrical stimulation (3 Hz, 240 shocks) was applied at this time there was no additional release (data not shown).

There was no significant difference between the effects of GYKI-53784 (20 μM) and vehicle (control) on the ischemia-evoked release of [^{3}H]noradrenaline (Fig. 2). In contrast, dizocilpine (3 μM) significantly reduced the effect of ischemia on the release of [^{3}H]noradrenaline (Fig. 3) and desipramine (30 μM) enhanced the release of [^{3}H]noradrenaline (Fig. 4a) produced by the ischemic conditions.

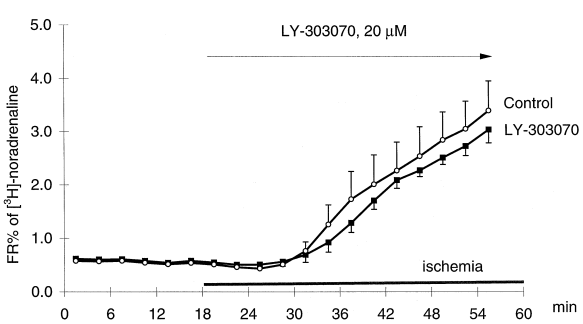

Fig. 2. Release of [^{3}H]noradrenaline in response to removal of oxygen and glucose (stimulation of ischemia) from the medium superfusing spinal cord slices of the rat. The tissue was exposed to ischemia from the 7th fraction till the end of the experiment (total 39 min). LY303070 (GYKI-53784) (20 μM) was added from the 4th fraction, 9 min before the induction of ischemia. There was no difference in the release between control (open circle, $n = 6$) and LY303070 (GYKI-53784)-treated slices (solid square, $n = 6$). FR, fractional release.

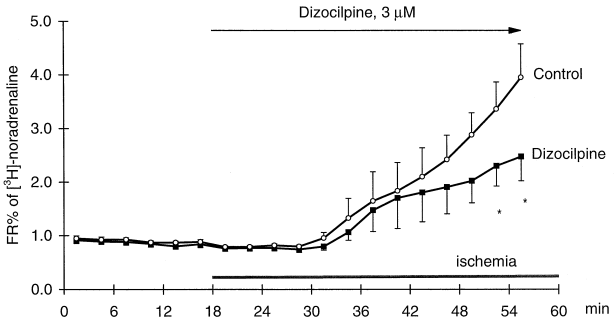

Fig. 3. Inhibitory effect of dizocilpine on the release of [^{3}H]noradrenaline in response to ischemia from the rat spinal cord. The slices were exposed to ischemia from the 7th fraction till the end of the experiment (total 39 min). Dizocilpine (3 μM) was added from the 4th fraction (9 min prior to ischemia) onward, as indicated. Note that dizocilpine prevented the effect of ischemia. Number of experiments: 6–6. Mean ± S.E.M. $^*P < 0.05$. FR, fractional release.

It is known that withdrawal of extracellular Ca^{2+} prevents intracellular Na^+ from being exchanged for extracellular Ca^{2+}, resulting in a faster accumulation of $[Na^+]_i$. In

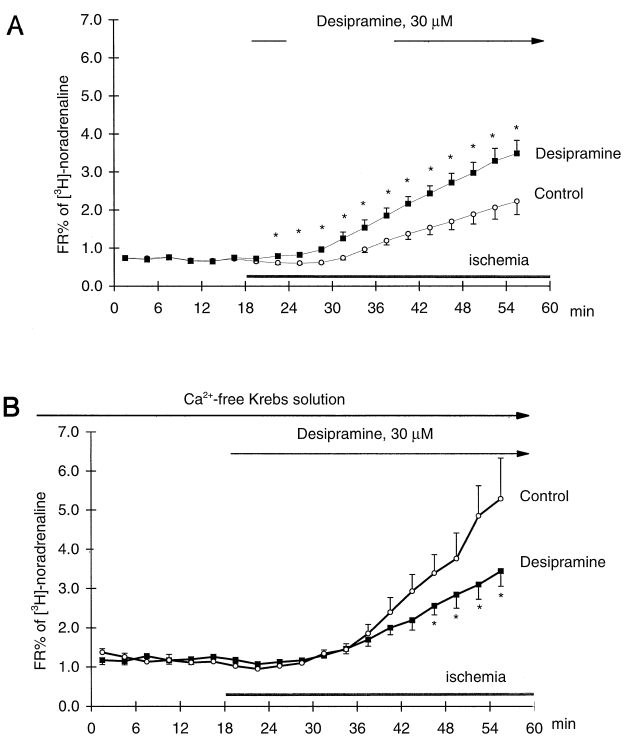

Fig. 4. Effect of desipramine on the excessive release of [^{3}H]noradrenaline from superfused rat spinal cord slices evoked by oxygen and glucose removal in the presence and absence of Ca^{2+} (ischemia). Ischemia was induced from the 7th fraction till the end of the experiment (total 39 min). (a) Effect of desipramine in the presence of extracellular Ca^{2+}. Desipramine (30 μM) was added from the 4th fraction (9 min prior to ischemia) onward and enhanced the release. (Open circle; control, $n = 7$, solid square; desipramine, $n = 7$.) (b) Effect of desipramine in the absence of extracellular Ca^{2+}. When Ca-free Krebs solution was applied, the resting release was more pronounced in the control group. Desipramine (30 μM) reduced the release of [^{3}H]noradrenaline. (Open circle; control, $n = 7$, solid square; desipramine, $n = 7$.) $^*P < 0.05$. FR, fractional release.

addition, the Na^+–Ca^{2+} exchange is an important mechanism of Ca^{2+} influx in causing for neuronal injury (LoPachin and Lehning, 1997). Therefore, the effect of desipramine was also studied on [^{3}H]noradrenaline release under ischemic conditions when extracellular calcium ions were withdrawn. In agreement with our earlier observations (Uchihashi et al., 1998), omission of $[Ca^{2+}]_o$ potentiated the effect of ischemia on noradrenaline release (Fig. 4b), but under this condition desipramine was able to prevent partly the excessive release of [^{3}H]noradrenaline in response to hypoxia combined with glucose removal.

3.3. Release of glutamate evoked by ischemic condition

The release of glutamate and aspartate was also measured in 3-min fractions by HPLC. Hypoxia combined with glucose removal gradually enhanced the release of both glutamate and aspartate from 305.0 ± 138 to 535.1 ± 51.0 and 232.1 ± 7.7 to 441.0 ± 38.2 pmol/mg/min n = 5–5, $P < 0.05$, respectively. Dizocilpine completely prevented the effect of ischemic conditions to increase amino acid release (Fig. 5a,b). Under normoxic conditions it failed to affect the release. Clonidine (3 μM), an α_2-adrenoceptor agonist, failed to affect the release of glutamate and aspartate produced by 30 min of hypoxia combined with glucose deprivation. The release of glutamate was 608.4 ± 54.5 in the absence and 584.7 ± 67.9 pmol/mg/min in the presence of clonidine (3 μM). The release of aspartate was also not reduced: it was 465.7 ± 31.8 in the absence and 439.7 ± 41.3 pmol/mg/min in the presence of clonidine.

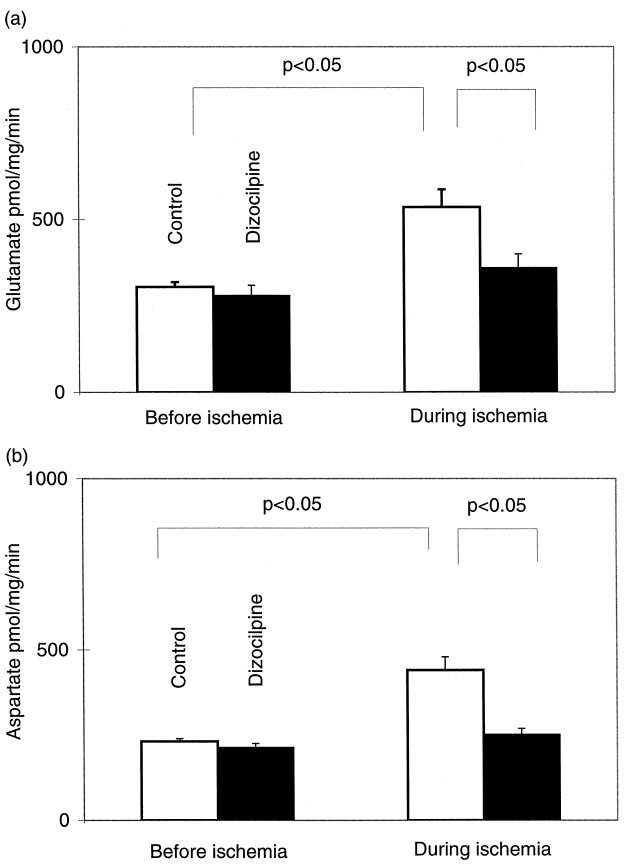

Fig. 5. Effect of dizocilpine (MK-801, 30 μM) on the ischemia-induced release of glutamate (a) and aspartate (b) from superfused rat spinal cord slices. Ischemia was introduced from the 7th fraction till the end of the experiment (total 39 min). In the control group (white column, $n = 5$), the release of glutamate and aspartate in 3-min functions were enhanced during ischemia (36–39 min from the onset of ischemia). Note that dizocilpine (3 μM) prevented the effect of ischemia to increase the release of these amino acids (black column, $n = 5$). The release of glutamate and aspartate in response to ischemia was $[Ca^{2+}]_o$ independent (data not shown). This is in agreement with the results of Ikeda et al. (1989) for the hippocampus.

4. Discussion

As with our earlier observations for dopamine release in the striatum (Milusheva et al., 1996) and noradrenaline release in the spinal cord (Uchihashi et al., 1998), the $[Ca^{2+}]_o$ dependent release of [^{3}H]noradrenaline from spinal cord slices associated with axonal conduction was completely blocked under ischemic conditions simulated by hypoxia and removal of glucose from the Krebs solution. However, the $[Ca^{2+}]_o$ independent resting release of noradrenaline and excitatory amino acids (glutamate and aspartate) was significantly increased by hypoxia combined with glucose removal. These findings are in agreement with those found for the hippocampus (Kauppinenen et al., 1988; Ikeda et al., 1989).

Dizocilpine, an NMDA receptor antagonist, completely antagonized the effect of ischemic conditions: but it potentiated the stimulation-evoked release. It seems likely that the latter effect of dizocilpine is associated with inhibition of noradrenaline uptake, because in the presence of desipramine, a noradrenaline uptake blocker, it failed to influence the release associated with axonal conduction. This explanation is supported by the finding that dizocilpine is able to inhibit noradrenaline uptake in the hippocampus (Snell et al., 1988) and vas deferens (Pubill et al., 1996). However, the effect of dizocilpine on ischemia-induced noradrenaline release is very likely related to its NMDA receptor antagonistic property and not its uptake-blocking action. While desipramine, an uptake blocker, potentiated the effect of ischemia (Fig. 4a), dizocilpine significantly reduced it (Fig. 3). This indicates that the uptake inhibitory action of dizocilpine is not involved, in its effect, on $[Ca^{2+}]_o$ independent release.

In our experiments with spinal cord slices, ischemic conditions also enhanced the release of glutamate and aspartate, and dizocilpine prevented the effect of ischemia. Ghribi et al. (1995) also showed that dizocilpine inhibits glutamate release evoked by ischemia in a microdialysis study of the striatum. Dizocilpine may be able to inhibit

the positive feedback modulation of glutamate release (Connick and Stone, 1988; Adamson et al., 1990; Martin et al., 1991, 1994), therefore, much less glutamate is released and consequently less noradrenaline is released via stimulation of NMDA receptors located on the noradrenergic axon terminals.

It has been assumed that excessive glutamate release contributes to secondary neuronal death after acute insult such as ischemia, hypoxia, hypoglycemia and trauma (Wieloch et al., 1985; Lobner and Lipton, 1990; Wrathall et al., 1992, 1994; Katoh et al., 1997). It is impossible to explain the whole event by excitatory amino acids alone, because there will be additional mechanisms such as the excessive release of other transmitters (Globus et al., 1988, 1989; Martin et al., 1998). It is unclear whether the excessive release of noradrenaline has any neurotoxic effect, but several authors have suggested that it does (Globus et al., 1989; Halonen et al., 1995). It has also been shown that adrenoceptors are involved in the activation of cytokine production (Bencsics et al., 1995; Haskó et al., 1995, 1998) that is also involved in the neurotoxic effects (e.g., tumor-necrosis factor-α). In this respect, it seems likely that an excessive release of noradrenaline may worsen the situation after acute insults, especially in spinal trauma, where the outcome is influenced by complex factors including massive neuronal discharge, edema and ischemia (Green et al., 1997).

The spinal descending noradrenergic system originates from the brain stem (cf. Zhang et al., 1997a), and noradrenaline released from axon terminals inhibits glutamate release via presynaptic α_2-adrenoceptors (Kamisaki et al., 1993; Schlicker and Göthert, 1998). In contrast, the ischemia-induced release of glutamate was not reduced. In addition, the presence of NMDA receptors in the spinal dorsal horn has been shown (Liu et al., 1994), as has the NMDA-receptor stimulated release of noradrenaline (Klarica et al., 1996) but not that of γ-aminobutiric acid (GABA), acetylcholine, glycine or serotonin (Klarica et al., 1996), indicating a strong linkage between adrenergic and glutamatergic systems.

The finding that dizocilpine inhibited noradrenaline release evoked by ischemia can be explained not only by its blockade of presynaptic NMDA receptors expressed on noradrenergic terminals, but also by its ability to suppress the increase in glutamate and aspartate release elicited by ischemia. Therefore, it is possible that prevention of noradrenaline release by excitatory amino acids is also an explanation.

It has been shown that dizocilpine improves neurological outcome (Green et al., 1995; Katoh et al., 1997). However, there is still controversy about the effectiveness of dizocilpine because of its hypothermic effect (Zhang et al., 1997b). The finding that dizocilpine under in vitro conditions suppressed the release of noradrenaline, glutamate and aspartate is certainly not due to the hypothermic effect of dizocilpine. Nevertheless, hypothermia is an effective neuroprotective mechanism against ischemic brain damage (Green et al., 1995), and it has been shown that hypothermia suppresses the non-exocytotic release of neurotransmitters (Rokkas et al., 1995; Zelles et al., 1995; Uchihashi et al., 1998; Vizi, 1998). It has also been shown that the neuroprotective effect of dizocilpine and hypothermia is additive (Green et al., 1995). Very recently it has been shown (Vizi, 1998) that low temperature inhibits the carrier-mediated release of transmitters without affecting the release associated with axonal conduction. In ischemia, it is assumed that the carrier-mediated noradrenaline uptake system works in the reverse direction (cf. Adam-Vizi, 1992). In the present study, desipramine potentiated the effect of ischemia on noradrenaline release, suggesting that dizocilpine acts in a different way from desipramine. However, desipramine suppressed the release when Ca^{2+} was withdrawn from the Krebs solution. It has been shown that under these conditions the non-vesicular release of neurotransmitters is increased (Zelles et al., 1995; Uchihashi et al., 1998; Vizi, 1998). The ischemia-evoked release is sensitive to sodium channel inhibitors (Uchihashi et al., 1998), suggesting that sodium influx contributes to the excessive release of noradrenaline. It is also known that sodium channel inhibitors have a neuroprotective effect (Breckwoldt et al., 1991; Kimura et al., 1998). Na^+ influx via Ca^{2+} channels can happen in the absence of Ca^{2+}, and the carrier-mediated uptake system depends on $[Na^+]_o$ (Adam-Vizi, 1992). In Ca^{2+}-free solution the Ca^{2+}/Na^+ exchange does not operate, and therefore $[Na^+]_i$ increases as Na^+ influx occurs via Ca^{2+} channels. Therefore, the moderation of the effects of ischemia by desipramine under conditions when $[Ca^{2+}]_o$ was withdrawn can be explained in the following way: desipramine inhibits Na^+ influx (Pancrazio et al., 1998) and thus reduces the rate of accumulation of $[Na^+]_i$, thereby preventing the excessive release of noradrenaline under ischemic conditions when Ca^+ ions are omitted. In the case of dizocilpine, the local anesthetic action, which would account for its effect on release, can be easily excluded. Dizocilpine failed to reduce or inhibit the stimulation-evoked release of noradrenaline (see Table 1), as local anesthetics do.

It has been shown (Wrathall et al., 1994) that non-NMDA receptor antagonists have neuroprotective effects in spinal trauma. The opening of sodium channels and sodium influx due to AMPA receptor activation causes spinal edema, thus worsening the effects of ischemia in the limited space of the vertebral canal (Wrathall et al., 1994). In the present study AMPA receptor antagonists failed to influence noradrenaline release evoked by either axonal stimulation or ischemia. LY300168 (GYKI-53655) and its (−)isomer LY303070 (GYKI-53784) are the strongest 2,3-benzodiazepine compounds, having selectivity for the AMPA receptor (Mike et al., 1996; Vizi et al., 1996, 1997). The present result shows that there is little interaction between AMPA receptors and ischemia-induced noradrenaline release in the spinal cord.

5. Conclusion

In conclusion, it seems likely that because descending noradrenergic neurons are a main inhibitory system (Kamisaki et al., 1993), and because NMDA is involved with wind-up phenomenon and neuropathic chronic pain (Davies and Lodge, 1987), in which enhanced glutamate transmission and decreased inhibition are suggested as the main mechanism (Dickenson et al., 1997), the inhibitory effect of dizocilpine on the excessive release of noradrenaline and excitatory amino acids (glutamate and aspartate) in response to ischemia may be beneficial in treating neuropathic chronic pain and spinal cord injury.

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