J. Pharm. Pharmacol. 1996, 48: 85–89
Received June 9, 1995
Accepted August 4, 1995

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3-Nitropropionic Acid Exacerbates [^{3}H]GABA Release Evoked by Glucose Deprivation in Rat Striatal Slices

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Abstract

3-Nitropropionic acid (3-NPA) is a metabolic poison that produces lesions of striatal intrinsic neurones such as γ-aminobutyric acid (GABA) neurones. This study was carried out to determine whether 3-NPA would impair the ability of striatal GABAergic neurones to withstand hypoglycaemic stress.

3-NPA (500 μM) did not affect [^{3}H]GABA release from striatal slices under normal (11 mM) glucose concentrations. When the glucose concentration was lowered to 0·3 mM, however, 3-NPA greatly potentiated the leakage of [^{3}H]GABA from the slices. Blockade of *N*-methyl-D-aspartate (NMDA) or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors with 1 μM 5-methyl-10,11-dihydro-5H-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801) or 10 μM 2,3-dihydroxy-6-nitro-7-sulphamoylbenzo[F]quinoxaline (NBQX), respectively, or a combination of both, had no effect. However, blockade of voltage-dependent sodium channels with tetrodotoxin totally antagonized the [^{3}H]GABA overflow induced by the combination of 3-NPA and hypoglycaemia. Riluzole (10 to 100 μM), a neuroprotective agent that stabilizes the inactivated state of the voltage-dependent sodium channel, also dose-dependently antagonized the increase in [^{3}H]GABA release induced by the combination of the two stresses.

An impairment in energy homeostasis in neurones has been proposed to be involved in certain neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease and Huntington's disease (Koroshetz et al 1991; Beal 1992; Beal et al 1993a,b). In particular, mitochondrial activity has been reported to be decreased in patients suffering from Huntington's disease (Brennan et al 1985; Mann et al 1990). Moreover, glucose use in the striatum of asymptomatic individuals at risk for Huntington's disease has been reported to be decreased (Mazziotta et al 1987). As reported by Ludolph et al (1991), a delayed dystonia and a selective basal ganglia lesion have been observed in people having accidentally ingested the fungal toxin 3-nitropropionic acid (3-NPA). This agent inhibits ATP synthesis and increases the formation of lactic acid and, upon systemic administration to rats, produces striatal lesions similar to those seen post-mortem in Huntington's disease patients, in particular, loss of GABAergic efferent neurones (Alston et al 1977; Beal et al 1993a; Brouillet et al 1993). It has been proposed that 3-NPA impairs neuronal energy homeostasis and subsequently sensitizes cells to the excitotoxic effects of glutamic acid (Hamilton & Gould 1987; Simpson & Isacson 1993). 3-NPA does not, however, affect the basal release of glutamic acid (Erecinska & Nelson 1994). The experiments in the present study were undertaken to develop an in-vitro model to study the effects of 3-NPA on striatal GABAergic neurones. We have previously shown that GABA release from hippocampal slices increases when the slices are deprived of glucose (Margaill et al 1992). Presumably, this increased release is due to decreased intracellular ATP concentrations and a subsequent impairment of the cell's ability to maintain the membrane potential, which thus drifts to depolarized potentials. We have now studied the influence of 3-NPA in such a model, and evaluated the ability of anti-excitotoxic compounds to modulate it. These agents include: the excitatory amino acid receptor antagonists MK-801 (5-methyl-10,11-dihydro-5H-dibenzo[*a*,*d*]cyclohepten-5,10-imine), a blocker of *N*-methyl-D-aspartate (NMDA) receptors, and NBQX (2,3-dihydroxy-6-nitro-7-sulphamoylbenzo[*F*]-quinoxaline), a blocker of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate receptors; a voltage-dependent sodium-channel blocker (tetrodotoxin); and riluzole (2-amino-6-trifluoromethoxy benzothiazole), a neuroprotective agent (Malgouris et al 1989, 1994; Pratt et al 1992; Boireau et al 1994a,b) that stabilizes the inactivated state of the voltage-dependent sodium channel (Benoit & Escande 1991; Hebert et al 1994). The mechanism of action of riluzole has been recently discussed (Hubert et al 1994).

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Materials and Methods

Male Sprague-Dawley rats (Iffa Credo, France), 500–700 g (6 to 8 months old), were housed in groups of 3 in a controlled environment with a 12-h light-dark cycle. Food and water were freely available. The in-vitro release of GABA was studied as previously described for hippocampal slices (Margaill et al 1992) with slight modifications. Briefly, rat striata were sliced into ribbons (0·3 × 0·3 mm) with a McIlwain tissue chopper and incubated for 15 min at 37°C in an oxygenated (95% O_2–5% CO_2) physiological medium composed of (mM): 120 NaCl, 3·5 KCl, 25 $NaHCO_3$, 1 NaH_2PO_4, 1·2 $MgSO_4$, 1·2 $CaCl_2$ and 11

glucose to which had been added 0·05 mM gabaculine, an inhibitor of GABA-T, 5 mM β-alanine (to prevent glial uptake) and 0·05 μM [^{3}H]GABA (1361 GBq mM, New England Nuclear). The tissue was rinsed and 1-mL aliquots containing approximately 15 mg tissue were transferred to superfusion chambers consisting of Millipore filters (Millex HA; 0·45 μm). After 30-min superfusion with or without 500 μM 3-NPA at 0·4 mL min^{-1}, 8-min fractions were collected directly into vials and the amount of radioactivity was determined by liquid scintillation spectrometry. In fraction 3 to fraction 5 (24 min), the physiological medium was replaced by medium containing a low concentration of glucose and, in some cases, the studied compounds. Overall, 10 fractions of superfusion were collected. The radioactivity remaining in the filter at the end of the superfusion was measured, and radioactivity was expressed as a percentage of the total radioactivity present in the ribbons at the beginning of each fraction. The results are given either in terms of percent release for each fraction, or (to compare more easily different experimental conditions or the responses to pharmacological agents), in terms of percent release for the 5th fraction, which was generally the fraction of maximal response.

In-vitro data are expressed as the mean ± s.e.m. Statistical significance of the difference between control and treated groups was assessed by analysis of variance followed by a Student's *t*-test. The concentration inhibiting by 50% the effect of 3-NPA on the overflow from the 5th fraction (IC50 value) was calculated by computer-assisted iterative non-linear regression analysis with the ENZFITTER software package. 3-NPA, tetrodotoxin and gabaculine (3-amino-2,3-dihydrobenzoic acid) were purchased from Sigma Chemical (France). MK-801 was purchased from Research Biochemical Inc. (Natick, USA). NBQX was synthesized by our own chemistry department.

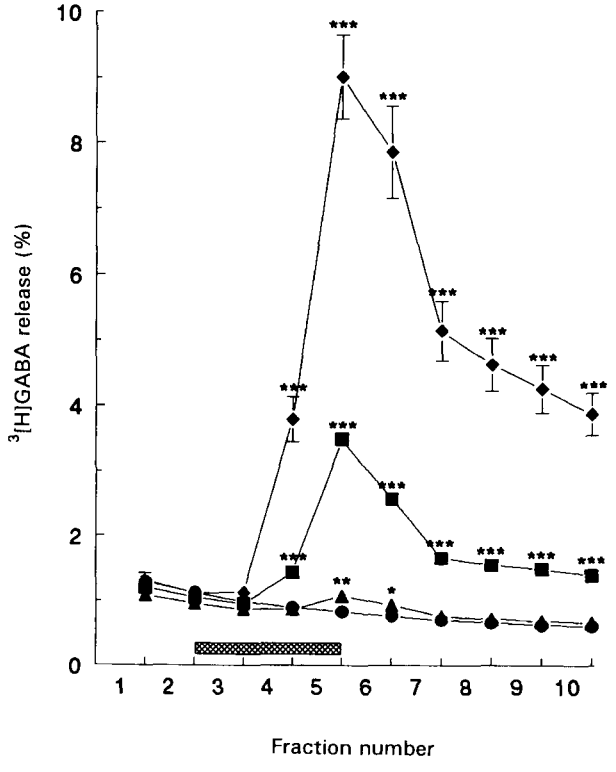

FIG. 1. Effects of decreasing glucose concentration on the release of [^{3}H]GABA from striatal slices. Basal release was determined in the presence of normal and reduced glucose concentration (● 11 mM; ▲ 0·3 mM; ■ 0·1 mM) or in the total absence of glucose (◆). * $P < 0·05$, ** $P < 0·01$ and *** $P < 0·001$ when compared to controls superfused with 11 mM glucose. Hatched box: modification of glucose in the medium. Each curve represents the mean of 6 to 21 individual data.

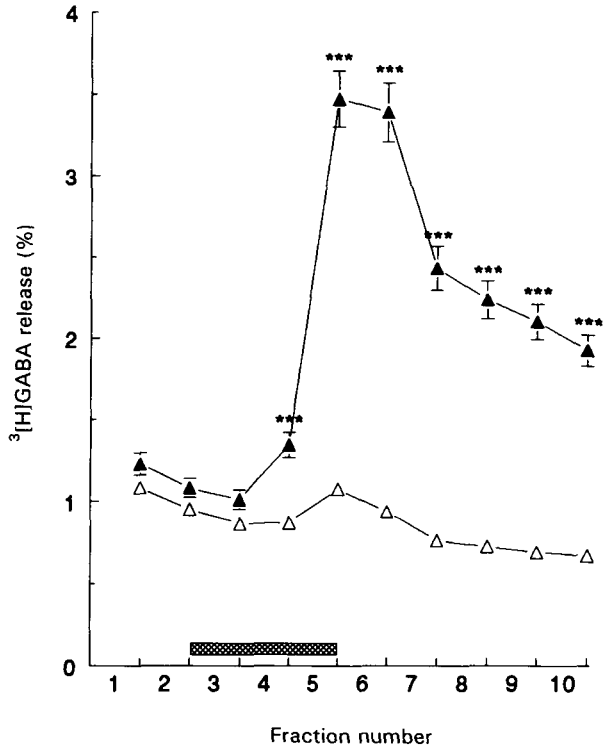

FIG. 2. Effects of 500 μM 3-NPA on the release of [^{3}H]GABA from striatal slices in the presence of 0·3 mM glucose. After 30 min of superfusion with or without 500 μM 3-NPA, 8-min fractions were collected. The fractions of superfusion with 0·3 mM glucose are indicated by the hatched box; otherwise, the glucose concentration was 11 mM. When added, 3-NPA was present throughout the superfusion. Each curve represents the mean of 6 to 21 individual data. 3-NPA: present (▲) or absent (△). *** $P < 0·001$ when compared to respective controls.

Results

For striatal ribbons bathed in medium containing glucose at a physiological concentration, the percent of [^{3}H]GABA released in the first 8-min fraction averaged 1·18 ± 0·03 (n = 50). The effect of lowering the glucose concentration is illustrated in Fig. 1. The basal release of [^{3}H]GABA increased when glucose concentration was lowered to 0·1 mM. In the total absence of glucose, the release of [^{3}H]GABA increased approximately 9 times. The 0·3 mM glucose concentration slightly increased the release of [^{3}H]GABA. Subsequently, when the glucose-poor medium was replaced with the physiological medium, the slices recovered partially from the hypoglycaemic (0 and 0·1 mM) periods. As the 0·3 mM glucose concentration was very close to the threshold of responsiveness of striatal slices, we mainly used this concentration for subsequent experiments. When slices were superfused with 500 μM 3-NPA in the

Table 1. Concentration-response to glucose in the presence or in the absence of 500 μM 3-NPA.

Glucose concn (mM)	[^{3}H]GABA release (%)	
	with 3-NPA (500 μM)	without 3-NPA
0·0	10·71 ± 0·84	9·00 ± 0·64
0·1	7·32 ± 0·22[a]	3.49 ± 0·12
0·3	3·46 ± 0·17[a]	1·07 ± 0·03
11·0	0·96 ± 0·07	0·83 ± 0·09

Values given are the means ± s.e.m. of 3 to 15 individual determinations of the radioactivity in the 5th fraction. [a] $P < 0.001$ when compared to respective controls.

presence of a normal glucose concentration, the release of [^{3}H]GABA was barely modified (see below). However, superfusion of 500 μM 3-NPA significantly increased the release of [^{3}H]GABA from rat striatal slices bathed in medium containing glucose at a concentration of 0·3 mM (Fig. 2). An increase in [^{3}H]GABA release obtained with 3-NPA was still observed (though less marked) for slices bathed in low glucose and then reperfused with glucose at physiological concentration (Fig. 2). The concentration-response to glucose in the presence or in the absence of 3-NPA is given in Table 1. Although 3-NPA did not modify [^{3}H]GABA release when glucose was present at normal concentrations, a potentiating effect was observed at a glucose concentration of 0·1 mM, whereas in the total absence of glucose, 3-NPA did not significantly modify the efflux of [^{3}H]GABA (Table 1).

Neither MK-801 (1 μM) nor NBQX (10 μM) antagonized the release of [^{3}H]GABA induced by 3-NPA in medium containing 0·3 mM glucose (Table 2). The combination of these two compounds was also inactive. Tetrodotoxin (1 μM) totally antagonized the increase in [^{3}H]GABA release (Table 2). Riluzole, at the concentrations tested (10, 30 and 100 μM), antagonized dose-dependently (IC50 = 38 μM) the increase in [^{3}H]GABA release from the slices (Fig. 3). The antagonistic effect of riluzole was reproduced when the concentration of glucose was 0·1 mM (data not shown). By itself, riluzole at the concentration of 100 μM decreased slightly (24%; n = 6) the release of [^{3}H]GABA in the presence of 0·3 mM glucose (data not shown).

Table 2. Effects of 1 μM tetrodotoxin, 1 μM MK-801, 10 μM NBQX and the combination of NBQX and MK-801 on the release of [^{3}H]GABA in striatal slices induced by 3-NPA (500 μM) in the presence of 0·3 mM glucose.

	[^{3}H]GABA release (%)
Control	1·04 ± 0·03
3-NPA	2·97 ± 0·22[a]
+ tetrodotoxin	1·21 ± 0·30[b]
+ MK-801	3·67 ± 0·11
+ NBQX	2·59 ± 0·39
+ NBQX + MK-801	2·89 ± 0·14

Values given are the mean ± s.e.m. of 3 to 15 individual determinations of the radioactivity in the 5th fraction. [a] $P < 0.001$ compared with control. [b] $P < 0.001$ compared with 3-NPA alone.

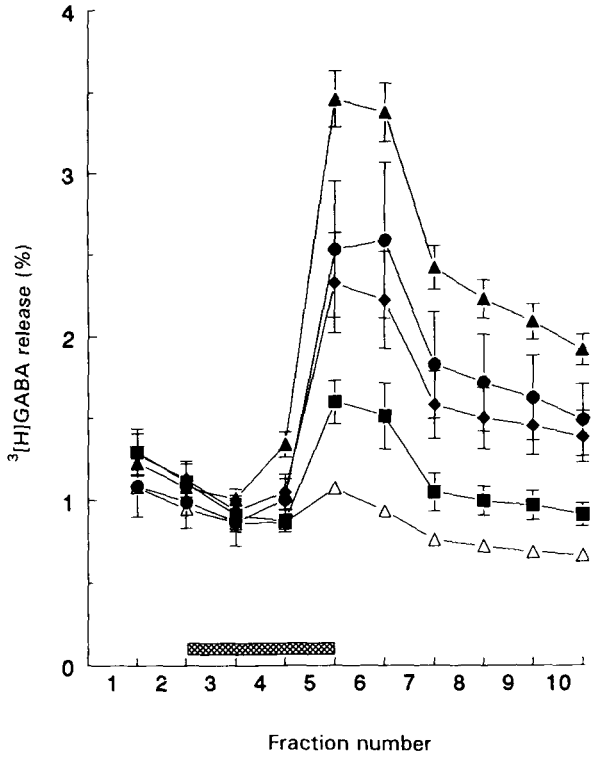

FIG. 3. Dose-effect of riluzole on the release of [^{3}H]GABA induced by continuous superfusion with 3-NPA (500 μM) in the presence of 0·3 mM glucose (hatched box). 3-NPA was added 30 min before the collection of fractions and maintained throughout the experiment. Basal release was determined in the presence (▲) or the absence (△) of 3-NPA. Riluzole (● 10 μM; ◆ 30 μM; ■ 100 μM) was added during the period of superfusion with glucose at modified concentrations. Each point represents the mean ± s.e.m. of 3 to 18 individual determinations.

Discussion

In our experiments, the release of [^{3}H]GABA from GABAergic neurones rose above basal levels in rat striatal slices in which energy had been depleted by a reduction in the supply of glucose. Thus, this increase in [^{3}H]GABA release may have been due to a decrease in ATP synthesis and to the consequent impairment of ATP-dependent processes (release; uptake; Na^+, K^+-ATPase) involved in the maintenance of proper neuronal activity. The superfusion of rat striatal slices with 500 μM 3-NPA, a toxin known to block the Krebs cycle and complex II of the mitochondrial chain (Ludolph et al 1991), did not on its own increase [^{3}H]GABA release. This observation of ours fits well with recent results showing that 3-NPA (1–2 mM) does not by itself alter the basal release of GABA (or that of glutamate and biogenic amines) from synaptosomes (Erecinska & Nelson 1994). However, we observed that slices superfused with 3-NPA released considerably more [^{3}H]GABA in response to hypoglycaemia. In the total absence of glucose, 3-NPA had no significant potentiating effect, possibly because the ability of the GABAergic neurones to release the neurotransmitter had already reached a maximum. It is interesting that 0·3 mM glucose was a concentration very close to the minimum tolerated by the neurones; below it, they did not maintain consistently their normal level of GABA release. Thus, we decided to use glucose at a

concentration of 0·3 mM for the pharmacological study. Interestingly, the potentiating effect of 3-NPA we observed can be compared with the results of Erecinska & Nelson (1994) who reported that this toxin increases the release of GABA (as well as that of glutamate, 5-hydroxytryptamine and dopamine) when depolarizing agents are present. From our results, we conclude that, if 3-NPA is present, GABA neurones are more sensitive to hypoglycaemia. An increase in [^{3}H]GABA release was still observed after reperfusion with glucose at a physiological concentration, suggesting that the impairment by the toxin of the GABA-releasing process was not totally reversible. We suggest that the lasting increase in [^{3}H]GABA release was due to the irreversible inhibition of succinate dehydrogenase. Moreover, these data also suggest that the combination of both stresses (3-NPA plus reduced glucose) may result in a greater reduction in ATP synthesis, and greater decrease in the efficiency of ATP-dependent processes, than would occur with either stress alone.

The ability of excitatory amino acid receptor antagonists to protect against 3-NPA neurotoxicity in-vivo is still open to debate (Beal et al 1993a). It was recently reported that the injection of 3-NPA in-vivo impairs the ability of neurones to resist insult from a local infusion of NMDA (Simpson & Isacson 1993). Furthermore, the neurotoxicity of glutamic acid is enhanced by disruption of the central energy supply (Novelli et al 1988). It is thus possible that in our experiments, excitotoxic insult caused by endogenous glutamic acid may have contributed to the deleterious effects of 3-NPA on [^{3}H]GABA release. Glutamergic neurotransmission in the rat striatum is mediated by both NMDA and AMPA/kainate receptors on GABAergic neurons. Blockade of these receptors with MK-801 or NBQX respectively did not, however, modify the stimulation of [^{3}H]GABA release by 3-NPA observed in this study. Our results indicate that the increase in [^{3}H]GABA release induced by 3-NPA and energy deprivation is not mediated by glutamate receptors. Interestingly, it has been proposed that 3-NPA-induced depolarization does not involve the stimulation of glutamate receptors (Riepe et al 1992). The increase in [^{3}H]GABA release that we observed could represent an early index of 3-NPA neurotoxicity.

We found that the stimulatory effect of 3-NPA could be abolished by incubation with tetrodotoxin, a blocker of voltage-dependent sodium channels. This suggests that the enhancement of transmitter release may be a consequence of depolarization due to the opening of sodium channels, rather than simply a leakage of the neurotransmitter. This hypothesis is supported by the observation that riluzole, which stabilizes the inactivated state of the voltage-dependent sodium channel, also blocked the effect of 3-NPA. The activity of riluzole in this model is presumably due to its effects at sodium channels, rather than to its effects on glutamergic transmission. Furthermore, this putative neuroprotective agent, as well as others responding under our experimental conditions, should be tested in other models of 3-NPA neurotoxicity.

In conclusion, the present study demonstrates that 3-NPA, which in animal models causes a neurotoxicity similar to that observed in idiopathic Huntington's disease in man, increases the release of [^{3}H]GABA from rat striatal slices submitted to hypoglycaemic conditions. Riluzole, a previously reported neuroprotective agent, antagonizes this effect of 3-NPA.

Acknowledgement

We wish to thank Karen Pepper for help with revising the manuscript.

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