Journal of Neurochemistry
Lippincott Williams & Wilkins, Philadelphia

The Bacterial Endotoxin Lipopolysaccharide Causes Rapid Inappropriate Excitation in Rat Cortex

Yushan S. Wang and Thomas D. White

Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada

Abstract: There is mounting evidence that inflammation and associated excitotoxicity may play important roles in various neurodegenerative disorders, such as bacterial infections, Alzheimer's disease, AIDS dementia, and multiple sclerosis. The immunogen *E. coli* lipopolysaccharide (LPS, endotoxin) has been widely used to stimulate immune/inflammatory responses both systemically and in the CNS. Here, we show that exposure of parietal cortical slices from adult rats to LPS triggered very rapid (<2.5 min) and sustained releases of the neurotransmitters glutamate and noradrenaline, and of the neuromodulator adenosine. The responses to LPS declined rapidly following removal of the LPS and exhibited no tachyphylaxis to repeated exposures to LPS. The detoxified form of LPS had no effect. LPS-evoked release of [^{3}H]noradrenaline, but not of glutamate or adenosine, appears to be partly due to the released glutamate acting at ionotropic receptors on the noradrenergic axons present in the cortical slices. LPS appears to release glutamate, which then acts at non-NMDA receptors to remove the voltage-sensitive Mg^{2+} block of NMDA receptors, thus permitting NMDA receptors to be activated and noradrenaline release to proceed. It seems possible that rapid, inappropriate excitation may occur in the immediate vicinity of gram-negative bacterial infections in the brain. If similar inappropriate excitations are also triggered by those immunogens specifically associated with Alzheimer's disease (β-amyloid), AIDS dementia (gp120 and gp41), or multiple sclerosis (myelin basic protein), they might explain some of the acute, transient neurological and psychiatric symptoms associated with these disorders. **Key Words:** Lipopolysaccharide—Excitation—NMDA—Glutamate—Adenosine—Noradrenaline—Rat parietal cortex.
J. Neurochem. **72,** 652–660 (1999).

In the past, the CNS was considered isolated and protected from most immune/inflammatory processes. However, there is mounting evidence that the CNS is affected by both central and peripheral inflammatory reactions (De Vries et al., 1997; Xiao and Link, 1998). Indeed, many neurodegenerative disorders, including Alzheimer's disease, AIDS dementia, and multiple sclerosis, as well as neurological pathologies associated more directly with specific bacterial infections of the CNS, appear to involve inflammatory processes (Perry et al., 1993; Yeung et al., 1995; Feuerstein et al., 1997). In many cases, it is not clear whether central inflammation is the cause of, or a response to, damage in the CNS. There is little doubt that neuronal cell death can trigger an inflammatory response in the brain. However, activation of an inflammatory response in the brain also has the capacity to cause pathological responses, including neuronal damage (Mattson and Mark, 1996).

Although clearly less robust than the peripheral inflammatory responses, the brain does possess its own inflammatory system. Microglia, which are largely quiescent (inactive), can be activated to perform macrophage-like functions. Astrocytes can also become reactive and possess characteristics of peripheral macrophages (Perry et al., 1993). The bacterial endotoxin lipopolysaccharide (LPS) has been widely used to stimulate the immune/inflammatory response both systemically and in the CNS in vivo and in vitro (Wan et al., 1994; Montero-Menei et al., 1996). Significantly, LPS activates macrophages to release various cytokines, which participate in inflammatory responses (Zhao and Schwartz, 1998). There is evidence that LPS may promote glutamate release in the CNS (Wan et al., 1994; al-Shabanah et al., 1996). In the present study, we show that LPS triggers the rapid releases of the neurotransmitters glutamate and noradrenaline (NA) and of the neuromodulator adenosine from slices of rat parietal cortex. Adenosine may function as a neuroprotectant in the CNS (Cronstein, 1995).

MATERIALS AND METHODS

Materials

LPS (from *Escherichia coli*, serotype 0127:B8), detoxified LPS (from *Escherichia coli*, serotype 0127:B8, delipidized by

Received August 21, 1998; revised manuscript received September 21, 1998; accepted September 22, 1998.

Address correspondence and reprint requests to Dr. T. D. White at Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

Abbreviations used: APV, D(−)-2-amino-5-phosphonopentanoic acid; LPS, lipopolysaccharide, endotoxin; MK-801, dizocilpine maleate; NA, noradrenaline; NBQX, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzo[*f*]quinoxaline-7-sulfonamide disodium.

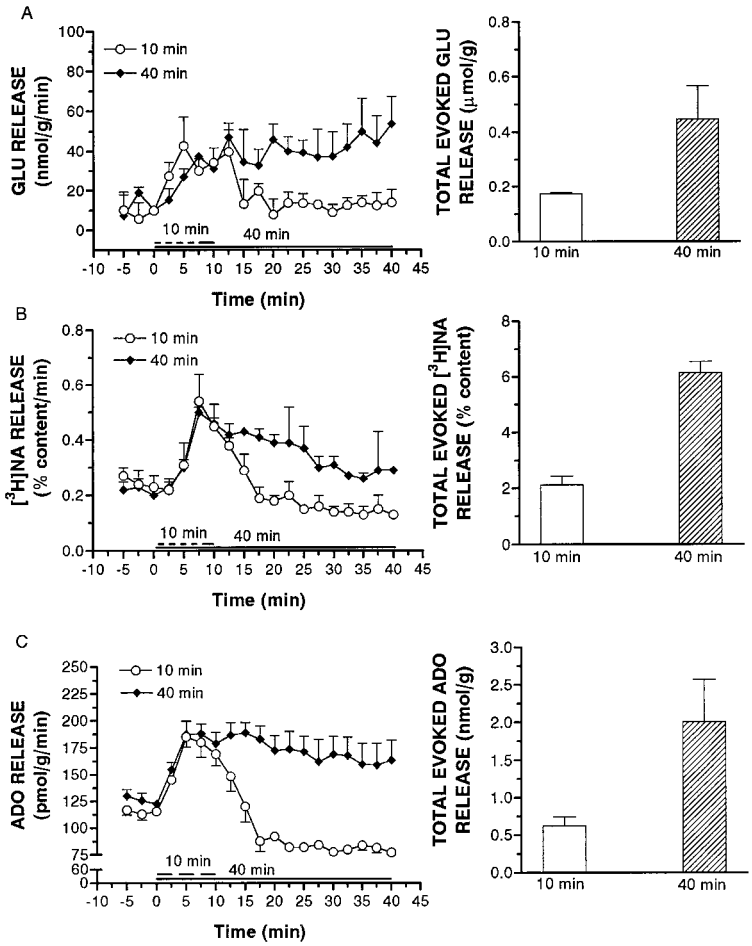

FIG. 1. LPS (50 μg/ml) triggers rapid (<2.5 min) releases of glutamate, [^{3}H]NA, and adenosine from slices of rat parietal cortex in the presence of extracellular Mg^{2+}. **A:** Release of glutamate (GLU). **B:** Release of [^{3}H]NA. **C:** Release of adenosine (ADO). Left panels show the time courses for LPS-evoked releases. Stimulation with LPS for 40 min induced rapid releases, which reached maximums at 7.5 min after exposure and persisted during exposure to LPS. The rates of release of glutamate, [^{3}H]NA, and adenosine rapidly returned to basal levels when LPS was withdrawn after 10 min of exposure. Right panels represent total evoked releases. Values are means ± SEM from four experiments.

alkaline hydrolysis), 1-octanesulfonic acid, and glutamate were purchased from Sigma (St. Louis, MO, U.S.A.). Dizocilpine maleate (MK-801), D(−)-2-amino-5-phosphonopentanoic acid (APV), and 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzo[*f*]quinoxaline-7-sulfonamide disodium (NBQX) were obtained from Research Biochemicals Inc. (Natick, MA, U.S.A.). L-[7-^{3}H]NA, Solvable, and Ecolite were purchased from Du Pont–New England Canada Inc. (Markham, Ontario, Canada). All other chemicals were obtained from commercial sources. All solutions were made in H_2O that had been distilled and then passed through a Millipak 40 sterile purification system. MK-801 was initially dissolved in ethanol and then diluted 1,000-fold in Krebs–Henseleit medium. In experiments involving MK-801, controls were superfused with an identical concentration of ethanol. All other drugs were dissolved in Krebs–Henseleit medium.

Preparation and superfusion of cortical slices

All procedures involving animals were approved by the Dalhousie University Animal Care Committee according to the guidelines of the Canadian Council on Animal Care. Adult male Sprague–Dawley rats (250–350 g; Charles River Canada, St. Constant, Quebec, Canada) were killed by decapitation in a guillotine. Slices (0.4 mm) of parietal cortex were prepared on a McIlwain tissue chopper and rapidly placed into ice-cold Krebs–Henseleit bicarbonate medium containing the following (in m*M*): NaCl, 111; $NaHCO_3$, 26.2; NaH_2PO_4, 1.2; KCl, 4.7; $CaCl_2$, 1.8; $MgCl_2$, 1.2; and glucose, 11; gassed with 95% O_2/5% CO_2 to maintain a pH of 7.4. Adjacent slices were placed alternately into each of two tissue baths so that each bath contained six slices from both sides of the brain, weighing a total of ~80 mg. The slices rested on nylon mesh screens in tissue baths adjusted to 0.5 ml as described previously (Hoehn and White, 1990*a*) and were immersed in a circulating water bath at 36°C. After an initial 5-min superfusion period, slices were labeled with [^{3}H]NA by superfusion for 10 min with oxygenated Krebs–Henseleit bicarbonate medium containing freshly prepared 10^{-7} *M* [^{3}H]NA (L-[^{3}H]NA specific activity 13.1 Ci/mmol) at 36°C. Superfusion was continued with Krebs–Henseleit bicarbonate medium for a further 90 min before collection of 24 serial 2.5-min fractions. After collection of three samples to determine basal release, the superfusing medium was switched to medium containing LPS for either 10 or 40 min, after which the superfusing buffer was switched back to normal Krebs–Henseleit buffer and the collection continued until the end of the experiment. For studies with two identical periods of stimulation, slices were first exposed to LPS for an initial 5 min after the basal collection of samples, the slices were allowed to recover in normal Krebs–Henseleit buffer for 30 min, and the second stimulation by LPS was delivered for another 5 min. Sample collection continued for 10 min after the

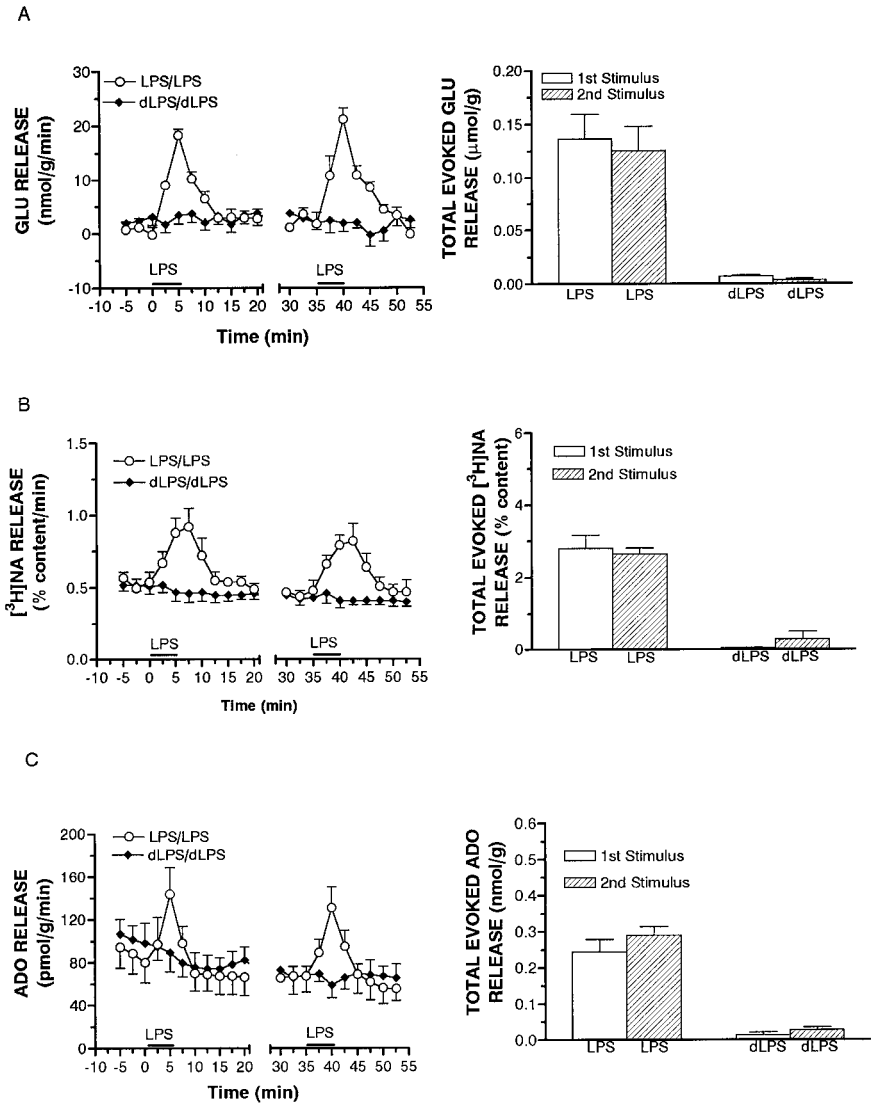

FIG. 2. Effect of LPS (50 μg/ml) and detoxified LPS (50 μg/ml) on glutamate, [^{3}H]NA, and adenosine release from slices of rat parietal cortex. **A:** Release of glutamate (GLU). **B:** Release of [^{3}H]NA. **C:** Release of adenosine (ADO). Left panels show the time courses for release after repeated exposure to LPS for 5 min each. No tachyphylaxis was observed for LPS-evoked releases of glutamate, NA, and adenosine. Detoxified LPS (dLPS) caused no release. Right panels represent total evoked releases. There were no differences in total evoked releases between the first and the second exposures to LPS. Values are means ± SEM from three experiments.

second stimulation. For glutamate receptor characterization studies, various receptor antagonists were introduced into the superfusing buffer 20 min before the second stimulation.

Detection of adenosine release

Samples (0.5 ml) of superfusate were deproteinated with $Ba(OH)_2$ and $ZnSO_4$ and then reacted with chloroacetaldehyde to form 1-N^6-ethenoadenosine, which was assayed using HPLC with fluorescence detection (Craig and White, 1992). Adenosine standards in Krebs–Henseleit medium were treated identically to the samples, and the amount of adenosine in the samples was quantified by comparison of peak heights with the standards. Evoked release was obtained by subtracting the basal rate of release immediately preceding exposure to LPS from every other sample. The total amount of evoked adenosine release was the amount released during the entire period of each exposure to LPS and was expressed as nanomoles per gram of cortex.

Detection of [^{3}H]NA release

After removal of the 0.5 ml of the superfusate for determination of adenosine, 0.5 ml of sample was placed into scintillation vials containing 10 ml of Ecolite and the disintegrations per minute of [^{3}H]NA released was determined with a Beckman model LS5801 scintillation counter. The slices were weighed and then solubilized in 1 ml of Solvable. Tissue [^{3}H]NA contents were determined by scintillation spectrometry in 14 ml of Ecolite. The rate of [^{3}H]NA release was standardized as the percentage of tissue content released per minute. The rate of evoked [^{3}H]NA release was obtained by subtracting the basal rate of release in the sample immediately preceding exposure to LPS from every other sample and was expressed as percentage of content released per minute. Total evoked [^{3}H]NA release was determined as the percentage of [^{3}H]NA content released during the entire period of exposure to LPS (Hoehn et al., 1990).

Detection of glutamate release

Glutamate content in the superfusing medium was measured by a specific fluorescence assay (excitation and emission at 335 and 430 nm, respectively) performed in a Perkin–Elmer fluorescence spectrophotometer (Nicholls and Sihra, 1986). Samples of superfusate (0.25 ml) were transferred to a reaction mixture containing glutamate dehydrogenase (40 U/ml) and

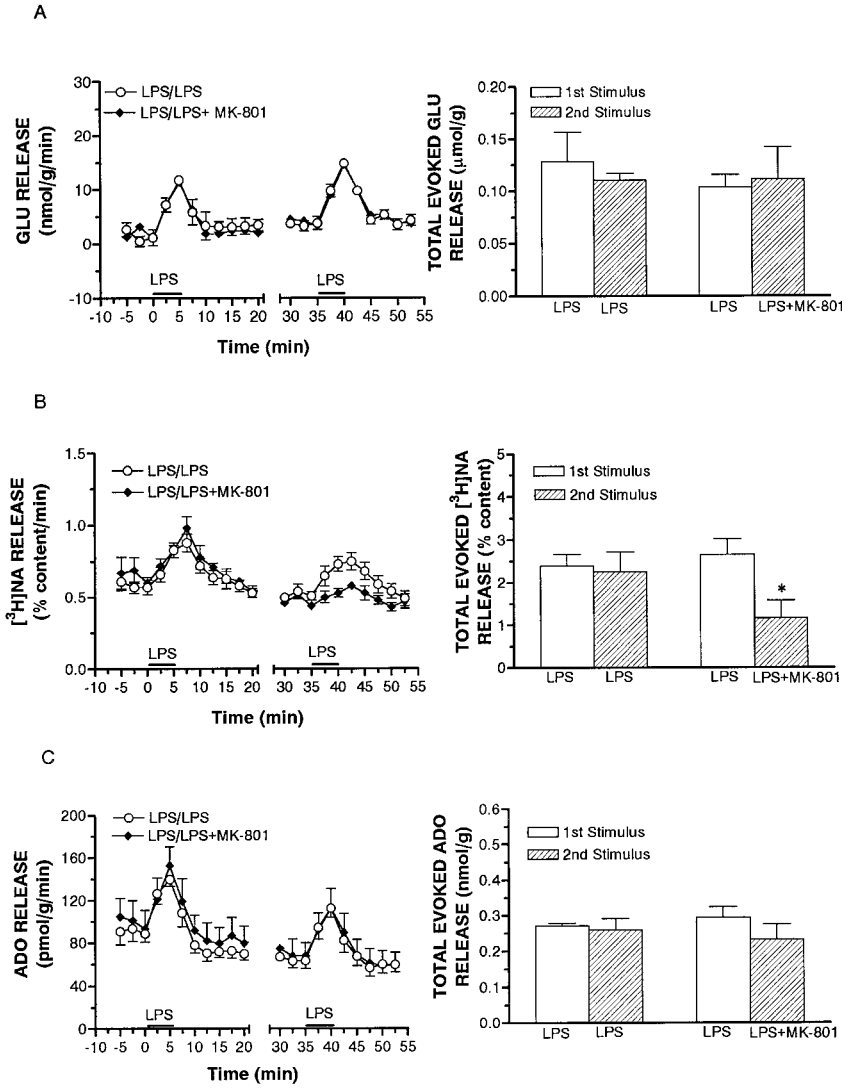

FIG. 3. Effect of the noncompetitive NMDA receptor antagonist MK-801 (3 μM) on LPS (50 μg/ml)-evoked glutamate, [^{3}H]NA, and adenosine release from slices of rat parietal cortex. **A:** Release of glutamate (GLU). **B:** Release of [^{3}H]NA. **C:** Release of adenosine (ADO). MK-801 significantly decreased the release of [^{3}H]NA evoked by LPS, but had no effect on the releases of glutamate or adenosine. Left panels show the time courses for releases after repeated exposure to LPS for 5 min each. Right panels represent total evoked releases after LPS stimulation. Values are means ± SEM from four experiments. *$p < 0.05$ compared with the first stimulation (LPS alone), Student's paired t test.

$NADP^+$ (1 mM) in HEPES buffer (in mM: NaCl, 123; KCl, 4.7; NaH_2PO_4, 1.2; $CaCl_2$, 1.8; $MgCl_2$, 1.2; glucose, 11; and HEPES, 10, pH 7.4). Glutamate oxidation by glutamate dehydrogenase to α-ketoglutarate led to the formation of NADPH with fluorescence emission. The rate of glutamate release (nanomoles per gram per minute) was calculated by referring to standard curves constructed for various concentrations of glutamate (in the linear range of 0.25–4.0 nM). Total evoked glutamate release was obtained by summing the total amount of glutamate released during the entire period of exposure to LPS, expressed as micromoles per gram.

Statistical analysis

Paired Student's t tests were conducted on the total glutamate, adenosine, and [^{3}H]NA releases from the two treatment groups.

RESULTS

LPS triggers the rapid releases of glutamate, [^{3}H]NA, and adenosine from cortical slices

LPS (1, 5, and 10 μg/ml) did not release the neurotransmitters glutamate and NA or the neuromodulator adenosine from slices of rat parietal cortex (data not shown). LPS (20 μg/ml) evoked very slight and inconsistent releases of glutamate, NA, and adenosine (data not shown). LPS (50 μg/ml) triggered very rapid (<2.5 min) and sustained releases of glutamate, [^{3}H]NA, and adenosine from slices of rat parietal cortex (Fig. 1). Their releases reached peak values within 7.5 min after exposure to LPS and were sustained while LPS was present in the superfusion medium (Fig. 1, 40-min treatment). Upon removal of LPS, the releases of glutamate, [^{3}H]NA, and adenosine rapidly returned to their basal levels (Fig. 1, 10-min treatment), indicating that these responses are readily reversible. The slices responded to repeated exposures to LPS without any apparent tachyphylaxis (Fig. 2). Finally, the detoxified form of LPS failed to release glutamate, [^{3}H]NA, or adenosine (Fig. 2), suggesting that LPS exerts its actions specifically through the biologically active, lipid portion of the molecule.

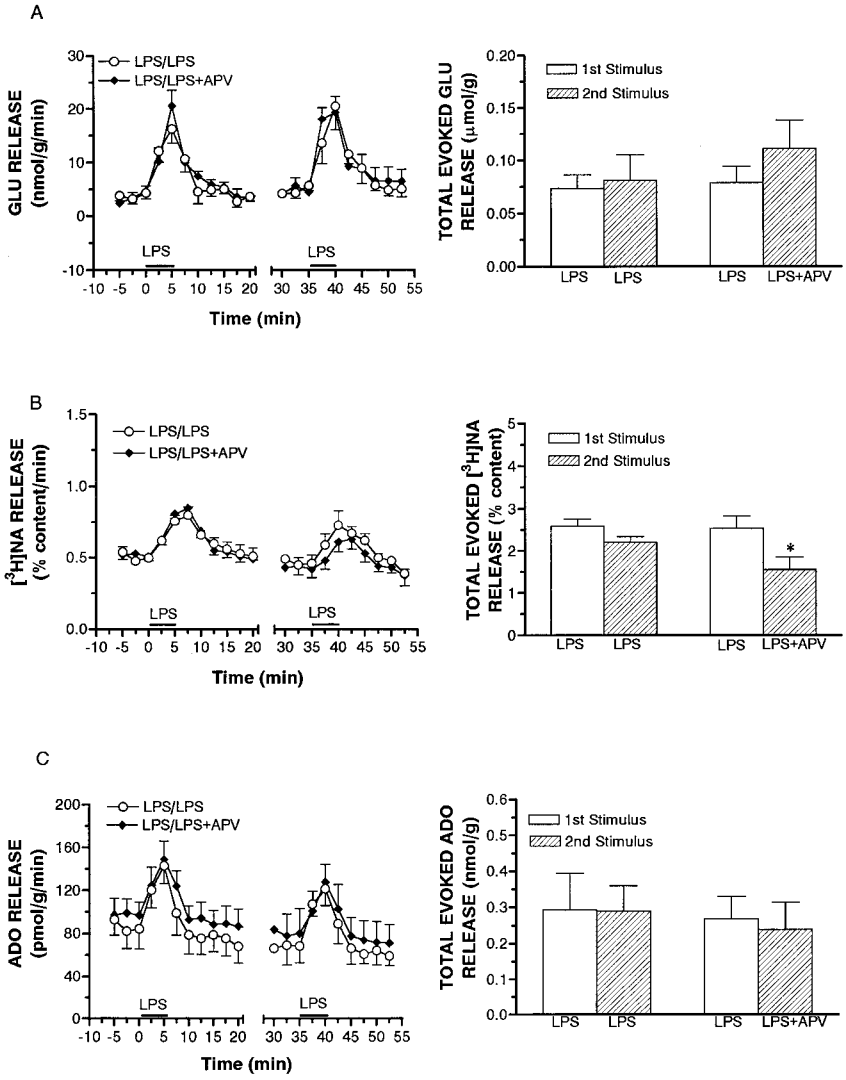

FIG. 4. Effect of the competitive NMDA receptor antagonist APV (500 μM) on LPS (50 μg/ml)-evoked glutamate, [^{3}H]NA, and adenosine release from slices of rat parietal cortex. **A:** Release of glutamate (GLU). **B:** Release of [^{3}H]NA. **C:** Release of adenosine (ADO). APV significantly decreased the release of [^{3}H]NA evoked by LPS, but had no effect on the releases of glutamate and adenosine. Left panels show the time courses for releases after repeated exposure to LPS for 5 min each. Right panels represent total evoked releases after LPS stimulation. Values are means ± SEM from four experiments. $^*p < 0.05$ compared with the first stimulation (LPS alone), Student's paired *t* test.

Role of NMDA and non-NMDA ionotropic receptors in LPS-evoked releases of glutamate, [^{3}H]NA, and adenosine from rat cortical slices

Activation of either NMDA or non-NMDA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate and kainate) receptors rapidly releases both NA and adenosine from brain slices (Hoehn and White, 1990*a*,*b*; Craig and White, 1993; Wang and White, 1998). This raised the possibility that the LPS-evoked releases of NA and adenosine might result from released glutamate acting at its receptors. Therefore, we examined the effects of the noncompetitive NMDA receptor antagonist MK-801 (3 μM) and a high concentration of the competitive NMDA antagonist APV (500 μM) on LPS-evoked releases of glutamate, [^{3}H]NA, and adenosine. LPS-evoked [^{3}H]NA release was decreased significantly by both MK-801 (Fig. 3B) and APV (Fig. 4B). However, neither MK-801 nor APV significantly reduced LPS-evoked releases of glutamate (Figs. 3A and 4A) or adenosine (Figs. 3C and 4C), indicating that these release processes are not likely mediated by the activation of NMDA receptors.

Significantly, LPS-evoked [^{3}H]NA release was observed even in the presence of a physiological concentration of Mg^{2+} (1.2 mM), a situation that we have shown previously abolishes the NMDA-evoked release of NA (Hoehn et al., 1990). The most likely explanation for this is that LPS releases glutamate, which then acts at non-NMDA receptors to relieve the Mg^{2+} block of NMDA receptors and thus permits NMDA receptor activation to take place. To test this hypothesis, we studied the effect of the non-NMDA receptor antagonist NBQX (10 μM) on LPS-evoked releases of glutamate, [^{3}H]NA, and adenosine. In the presence of Mg^{2+}, NBQX reduced the LPS-evoked release of [^{3}H]NA by ~50% (Fig. 5B). However, in the absence of Mg^{2+}, LPS-evoked release of [^{3}H]NA was not decreased significantly by NBQX (Fig. 5B). Finally, the combined blockade of both NMDA and non-NMDA receptors with MK-801 and

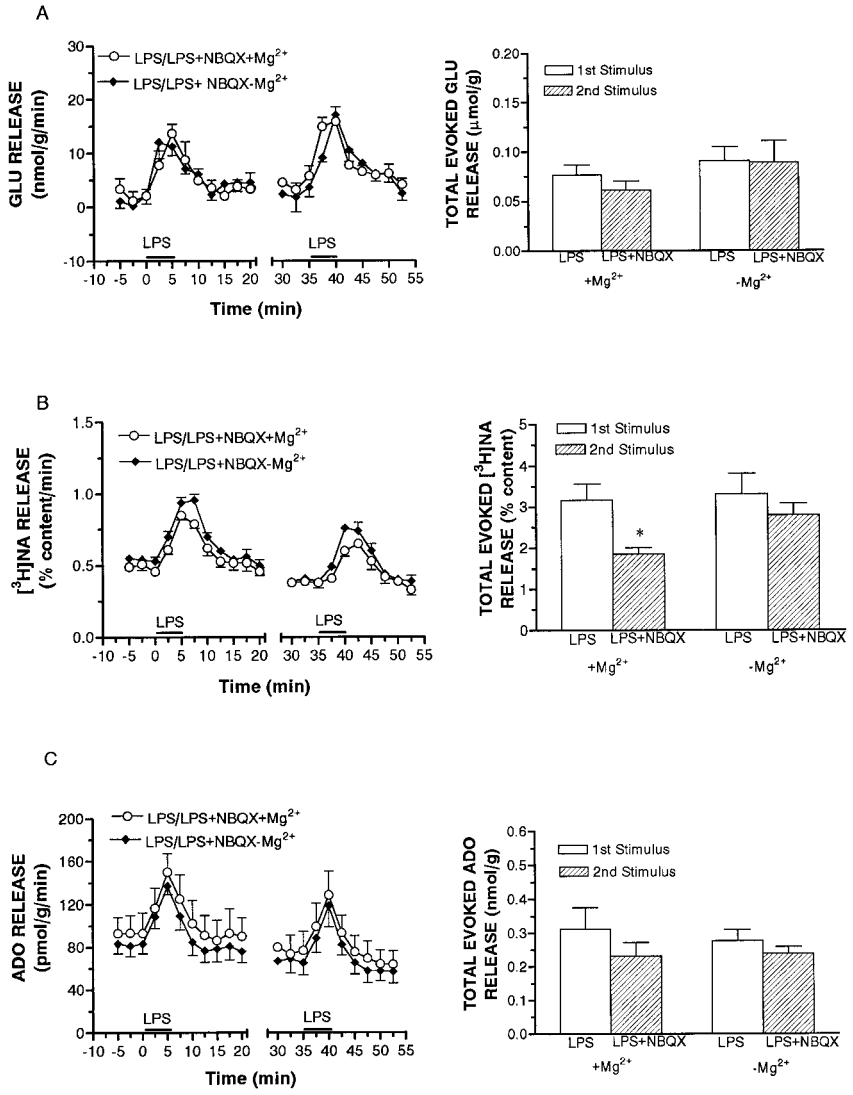

FIG. 5. Effect of the non-NMDA receptor antagonist NBQX (10 μM) on LPS-evoked glutamate, [^{3}H]NA, and adenosine releases from slices of rat parietal cortex in the presence and absence of extracellular Mg^{2+}. **A:** Release of glutamate (GLU). **B:** Release of [^{3}H]NA. **C:** Release of adenosine (ADO). In the presence of extracellular Mg^{2+}, NBQX significantly decreased the total LPS-evoked release of [^{3}H]NA, but had no effect on the releases of glutamate or adenosine. In the absence of extracellular Mg^{2+}, NBQX had no effect on LPS-evoked [^{3}H]NA release, nor did it affect the releases of glutamate or adenosine. Left panels show the time courses of LPS-evoked releases and the effects of NBQX. Right panels represent the total evoked releases. Values are means ± SEM from four experiments. $^*p < 0.05$ compared with the first stimulation (LPS alone), Student's paired *t* test.

NBQX, respectively, did not produce additive inhibition of LPS-evoked [^{3}H]NA release (Fig. 6B). NBQX had no effect on LPS-evoked glutamate (Fig. 5A) and adenosine (Fig. 5C) release either in the presence or in the absence of Mg^{2+}, nor did the combination of MK-801 and NBQX affect their releases (Fig. 6A and C).

DISCUSSION

The bacterial endotoxin LPS (50 μg/ml) triggered very rapid (<2.5 min) and sustained releases of glutamate, [^{3}H]NA, and adenosine from adult rat brain cortical slices. To the best of our knowledge, these are the most rapid responses to LPS ever observed in the brain (MacNeil et al., 1996; Klegeris and McGeer, 1997; Pistritto et al., 1998) and much too rapid to involve gene transcription and protein synthesis. The speed of onset of action is all the more remarkable when one considers that LPS has a molecular weight of $1–4 \times 10^6$ and would have difficulty penetrating the cortical slices. These rapid responses to LPS observed in the present study occurred in slices of supposedly normal, integrated parietal cortex from adult rats, rather than from cultures of neurons, glia, or arbitrary mixtures of the two, which may not reflect normal, integrated brain tissue. Nor were extraneous inflammatory cell types, which may not reside in normal, integrated brain tissue, introduced into the preparation (Skaper et al., 1996).

The concentration of LPS (50 μg/ml) used was relatively high. However, LPS concentrations in the immediate vicinity of gram-negative bacteria could be very high. In any event, the LPS-evoked responses observed in this study are unlikely to result from nonspecific effects or acute damage to brain cells for the following reasons. First, LPS-evoked releases of glutamate, NA, and adenosine were all readily reversible, rapidly returning to basal levels when LPS was withdrawn. Second, the slices responded to repeated exposures to LPS without any apparent tachyphylaxis. Finally, the detoxified

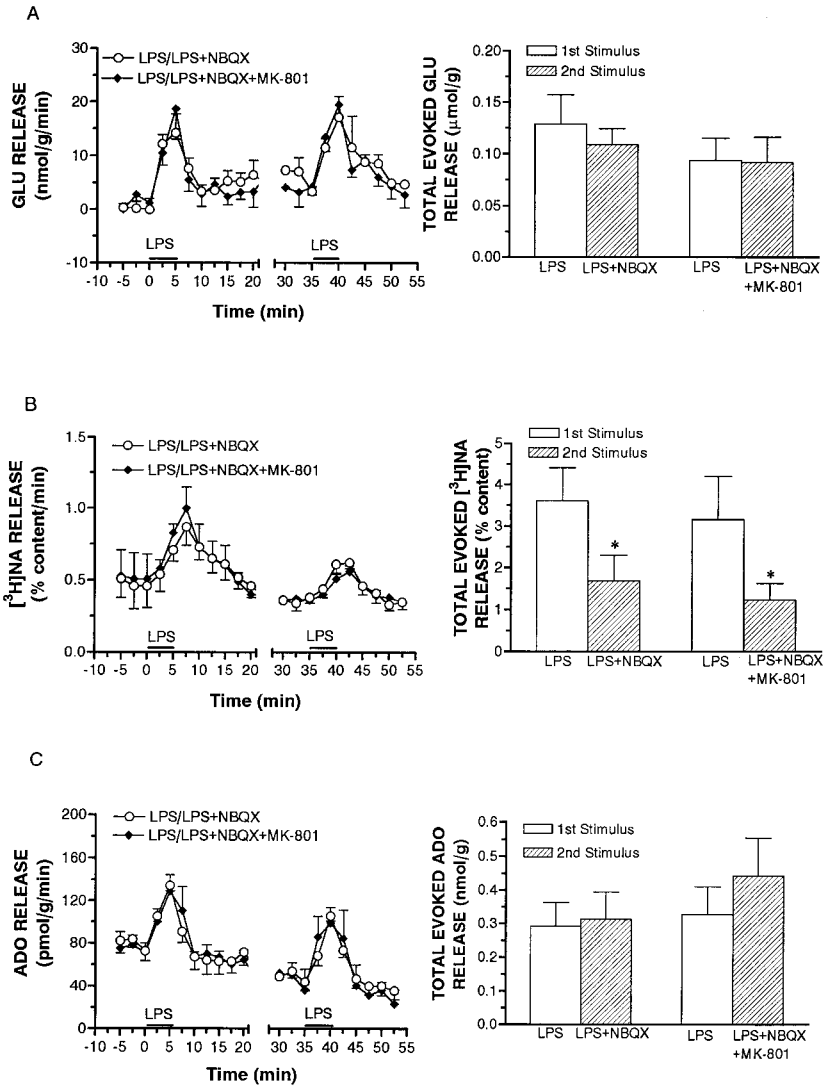

FIG. 6. Effect of the non-NMDA receptor antagonist NBQX (10 μM) and the combination of NBQX and MK-801 (3 μM) on LPS-evoked glutamate, [^{3}H]NA, and adenosine release from slices of rat parietal cortex in the presence of extracellular Mg^{2+}. **A:** Release of glutamate (GLU). **B:** Release of [^{3}H]NA. **C:** Release of adenosine (ADO). NBQX significantly decreased the total LPS-evoked release of [^{3}H]NA, but had no effect on the releases of glutamate or adenosine. The combination of the two antagonists did not produce additive inhibition of [^{3}H]NA release, nor did it reduce LPS-evoked releases of glutamate or adenosine. Left panels show the time courses of LPS-evoked releases and the effects of NBQX and MK-801. Right panels represent the total evoked releases. Values are means ± SEM from four experiments. $^*p < 0.05$ compared with the first stimulation (LPS alone), Student's paired *t* test.

form of LPS (50 μg/ml) failed to release glutamate, NA, or adenosine, suggesting that LPS exerts its actions specifically through the biologically active lipid portion of the molecule (Rietschel et al., 1996).

LPS-evoked release of [^{3}H]NA was decreased significantly by both the noncompetitive NMDA receptor antagonist MK-801 and by the competitive NMDA receptor antagonist APV, suggesting that the LPS-evoked release of [^{3}H]NA is partly due to the activation of brain NMDA receptors. This release occurred even when extracellular Mg^{2+} was present in the medium. Subsequent studies with non-NMDA receptor antagonists, alone and in combination with MK-801, indicate that LPS appears to release glutamate, which then acts at non-NMDA receptors to relieve the Mg^{2+} block of NMDA receptors present on noradrenergic axons, and thus permits NMDA receptor activation to occur. In contrast, neither NMDA nor non-NMDA ionotropic receptors appear to mediate LPS-evoked release of either glutamate or adenosine. In the case of adenosine, this is surprising because the activation of either NMDA or non-NMDA ionotropic receptors has been shown to release adenosine from cortical slices (Craig and White, 1993). It seems that LPS-evoked adenosine release involves processes other than those mediated by released glutamate. It is possible that LPS-evoked releases of glutamate and adenosine are mediated by other factors associated with immune/inflammatory responses, such as cytokines and/or prostanoids, which are released by LPS (Hua et al., 1996; Cannon et al., 1998; Martiney et al., 1998; Zhao and Schwartz, 1998).

It seems likely that the NA release evoked by LPS originates from noradrenergic axons present in the cortex. However, the cellular sites of origin for glutamate and adenosine release are much less clear. LPS-evoked release of glutamate from cultures of microglia has been reported previously (Patrizio and Levi, 1994), and the AIDS viral coat immunogen gp120 has been shown to promote the rapid release of glutamate from cultured astrocytes (Vesce et al., 1997). These findings raise the

possibility that the glutamate released in our study may have originated from glial, rather than neuronal, elements in the cortical slices.

The present study demonstrates a direct link between inflammation and rapid excitation in the cortex. Because of its inflammatory rather than neuronal origins, this excitation is inappropriate and hence pathological. The triggering of this inappropriate excitation, especially in the immediate vicinity of a gram-negative bacterial infection in the brain, could cause some of the transient but serious neurological/psychiatric symptoms associated with these infections. If similar rapid, inappropriate excitatory events are triggered by other immunogens, such as β-amyloid in Alzheimer's disease, HIV coat protein gp120 in AIDS dementia, and myelin basic protein in multiple sclerosis, this could account for some of the acute, but transient, neurological and psychiatric symptoms observed with these disorders. In fact, there is evidence that gp120 does promote the activation of NMDA receptors in hypothalamic slices (Raber et al., 1996). In extreme cases, this excessive and inappropriate excitation might even lead to subsequent excitotoxicity and neurodegeneration (Mattson and Mark, 1996).

The observation that LPS also triggers a rapid release of adenosine has important implications. Numerous studies have shown that adenosine, acting at specific adenosine A_{2A} receptors on macrophages and neutrophils, decreases inflammatory responses (Cronstein, 1995; Eigler et al., 1997). In addition, extracellular adenosine can act at neuronal A_1 receptors to decrease both the release of glutamate from neurons and glutamate's excitatory actions postsynaptically (Dolphin and Archer, 1983; Pasquini et al., 1987). Drugs that promote the formation of extracellular adenosine and/or potentiate its cellular actions may provide protection against this inappropriate excitation produced by immunogens in the brain.

Acknowledgment: The authors wish to thank Ms. Sharon Temple for her excellent technical assistance. This research was supported by grants to T.D.W. from the MRC of Canada and the Dalhousie University Medical Research Fund.

REFERENCES

al-Shabanah O. A., Mostafa Y. H., Hassan M. T., Khairaldin A. A., and al-Sawaf H. A. (1996) Vitamin E protects against bacterial endotoxin-induced increase of plasma corticosterone and brain glutamate in the rat. *Res. Commun. Mol. Pathol. Pharmacol.* **92,** 95–105.

Cannon T. R., Mann G. E., and Baydoun A. R. (1998) Mechanisms of acute vasodilator response to bacterial lipopolysaccharide in the rat coronary microcirculation. *Br. J. Pharmacol.* **123,** 637–644.

Craig C. G. and White T. D. (1992) Low-level *N*-methyl-D-aspartate receptor activation provides purinergic inhibitory threshold against further *N*-methyl-D-aspartate-mediated neurotransmission in the cortex. *J. Pharmacol. Exp. Ther.* **260,** 1278–1284.

Craig C. G. and White T. D. (1993) *N*-Methyl-D-aspartate- and non-*N*-methyl-D-aspartate-evoked adenosine release from rat cortical slices: distinct purinergic sources and mechanisms of release. *J. Neurochem.* **60,** 1073–1080.

Cronstein B. N. (1995) A novel approach to the development of anti-inflammatory agents: adenosine release at inflamed sites. *J. Invest. Med.* **43,** 50–57.

De Vries H. E., Kuiper J., De Boer A. G., Van Berkel T. J. C., and Breimer D. D. (1997) The blood–brain barrier in neuroinflammatory diseases. *Pharmacol. Rev.* **49,** 143–155.

Dolphin A. C. and Archer E. R. (1983) An adenosine agonist inhibits and a cyclic AMP analogue enhances the release of glutamate but not GABA from slices of rat dentate gyrus. *Neurosci. Lett.* **43,** 49–54.

Eigler A., Greten T. F., Sinha B., Haslberger C., Sullivan G. W., and Endres S. (1997) Endogenous adenosine curtails lipopolysaccharide-stimulated tumour necrosis factor synthesis. *Scand. J. Immunol.* **45,** 132–139.

Feuerstein G. Z., Wang X., and Barone F. C. (1997) Inflammatory gene expression in cerebral ischemia and trauma: potential new therapeutic targets. *Ann. NY Acad. Sci.* **825,** 179–193.

Hoehn K. and White T. D. (1990*a*) Role of excitatory amino acid receptors in K^+- and glutamate-evoked release of endogenous adenosine from rat cortical slices. *J. Neurochem.* **54,** 256–265.

Hoehn K. and White T. D. (1990*b*) *N*-Methyl-D-aspartate, kainate and quisqualate release endogenous adenosine from rat cortical slices. *Neuroscience* **39,** 441–450.

Hoehn K., Craig C. G., and White T. D. (1990) A comparison of *N*-methyl-D-aspartate-evoked release of adenosine and [^{3}H]noradrenaline from rat cortical slices. *J. Pharmacol. Exp. Ther.* **255,** 174–181.

Hua X. Y., Chen P., Fox A., and Myers R. R. (1996) Involvement of cytokines in lipopolysaccharide-induced facilitation of CGRP release from capsaicin-sensitive nerves in the trachea with interleukin-1β and tumor necrosis factor-α. *J. Neurosci.* **16,** 4742–4748.

Klegeris A. and McGeer P. L. (1997) β-Amyloid protein enhances macrophage production of oxygen free radicals and glutamate. *J. Neurosci. Res.* **49,** 229–235.

MacNeil B. J., Jansen A. H., Greenberg A. H., and Nance D. M. (1996) Activation and selectivity of splenic sympathetic nerve electrical activity response to bacterial endotoxin. *Am. J. Physiol.* **270,** R264–R270.

Martiney J. A., Cuff C., Litwak M., Berman J., and Brosnan C. F. (1998) Cytokine-induced inflammation in the central nervous system revisited. *Neurochem. Res.* **23,** 349–359.

Mattson M. P. and Mark R. J. (1996) Excitotoxicity and excitoprotection in vitro. *Adv. Neurol.* **71,** 1–35.

Montero-Menei C. N., Sindji L., Garcion E., Mege M., Couez D., Gamelin E., and Darcy F. (1996) Early events of the inflammatory reaction induced in rat brain by lipopolysaccharide intracerebral injection: relative contribution of peripheral monocytes and activated microglia. *Brain Res.* **724,** 55–66.

Nicholls D. G. and Sihra T. S. (1986) Synaptosomes possess an exocytotic pool of glutamate. *Nature* **321,** 772–773.

Pasquini J. M., Bizzozero O. A., Moreno M. B., and Soto E. F. (1987) Effects of calcium and cobalt ions on the transfer of proteins to the myeline membrane. *Neurochem. Int.* **11,** 17–22.

Patrizio M. and Levi G. (1994) Glutamate production by cultured microglia: differences between rat and mouse, enhancement by lipopolysaccharide and lack effect of HIV coat protein gp120 and depolarizing agents. *Neurosci. Lett.* **178,** 184–189.

Perry V. H., Andersson P. B., and Gordon S. (1993) Macrophages and inflammation in the central nervous system. *Trends Neurosci.* **16,** 268–273.

Pistritto G., Mancuso C., Tringali G., Perretti M., Preziosi P., and Navarra P. (1998) The relative contribution of constitutive and inducible cyclooxygenase activity to lipopolysaccharide-induced prostaglandin production by primary cultures of rat hypothalamic astrocytes. *Neuroscience* **246,** 45–48.

Raber J., Toggas S. M., Lee S., Bloom F. E., Epstein C. J., and Mucke L. (1996) Central nervous system expression of HIV-1 gp120 activates the hypothalamic–pituitary–adrenal axis: evidence for involvement of NMDA receptors and nitric oxide synthase. *Virology* **226,** 362–373.

Rietschel E. T., Brade H., Holst O., Muller-Loennies S., Mamat U., Zahringer U., Beckmann F., Seyde U., Brandenburg K., Ulmer A. J., Mattern T., Heine H., Schietter J., Loppnow H., Schonbeck U., Flad H. D., Hauschildt S., Schade U. F., Di Padova F., Kusumoto S., and Schumann H. D. (1996) Bacterial endotoxin: chemical constitution,

biological recognition, host response, and immunological detoxification, in *Pathology of Septic Shock* (Rietschel E. T. and Wagner H., eds), pp. 40–69, Springer Verlag, Berlin.

Skaper S. D., Facci L., Romanello S., and Leon A. (1996) Mast cell activation causes delayed neurodegeneration in mixed hippocampal cultures via the nitric oxide pathway. *J. Neurochem.* **66,** 1157–1166.

Vesce S., Bezzi P., Rossi D., Meldolesi J., and Volterra A. (1997) HIV-1 gp120 glycoprotein affects the astrocyte control of extracellular glutamate by both inhibiting the uptake and stimulating the release of the amino acid. *FEBS Lett.* **411,** 107–109.

Wan W., Wetmore L., Sorensen G. M., Greenberg A. H., and Nance D. M. (1994) Neural and biochemical mediators of endotoxin and stress-induced c-*fos* expression in the rat brain. *Brain Res. Bull.* **34,** 7–14.

Wang Y. and White T. D. (1998) Effect of protein kinase C activation on *N*-methyl-D-aspartate-evoked release of adenosine and [^{3}H]-noradrenaline from rat cortical slices. *J. Pharmacol. Exp. Ther.* **285,** 105–109.

Xiao B. G. and Link H. (1998) Immune regulation within the central nervous system. *J. Neurol. Sci.* **157,** 1–12.

Yeung M. C., Pulliam L., and Lau A. S. (1995) The HIV envelope protein gp120 is toxic to human brain-cell cultures through the induction of interleukin-6 and tumor necrosis factor-α. *AIDS* **9,** 137–143.

Zhao B. and Schwartz J. P. (1998) Involvement of cytokines in normal CNS development and neurological diseases: recent progress and perspectives. *J. Neurosci. Res.* **52,** 7–16.