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Endotoxin Administration Stimulates Cerebral Catecholamine Release in Freely Moving Rats as Assessed by Microdialysis

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In vivo microdialysis was used to measure changes in extracellular concentrations of catecholamines and indolamines in freely moving rats in response to administration of endotoxin (lipopolysaccharide, LPS). Dialysis probes were placed stereotaxically in either the medial hypothalamus or the medial prefrontal cortex. We used a repeated-measures design in which each rat received LPS or saline, and each subject was retested with the other treatment one week later. With the dialysis probes in the medial hypothalamus, intraperitoneal (ip) administration of LPS (5 μg) increased dialysate concentrations of norepinephrine (NE, 187%), dopamine (DA, 119%), and all their measured catabolites, except normetanephrine. Dialysate concentrations of NE and DA were elevated significantly in the fourth or fifth (20 min) collection period with a peak response at around 2 hr. They returned to baseline by about 4 hr. When the dialysis probes were placed in the medial prefrontal cortex, the same dose of LPS also elevated dialysate concentrations of NE and DA, but the increases were much smaller (ca. 20%). However, a dose of 100 μg LPS increased dialysate concentrations of NE and DA from the medial prefrontal cortex to an extent comparable to that of the 5 μg dose in the hypothalamus, and the response was more prolonged. Dialysate concentrations of serotonin could not be measured reliably, but those of its catabolite, 5-hydroxyindoleacetic acid (5-HIAA), were also elevated in both regions. The peak of 5-HIAA occurred at around 4 hr. Pretreatment of the rats with indomethacin (10 mg/kg ip) completely prevented the changes due to 100 μg LPS in the medial prefrontal cortex. These results support earlier neurochemical data suggesting that LPS stimulates the release of both DA and NE in the brain, and probably also release of serotonin.

Key words: endotoxin, indomethacin, microdialysis, dopamine, norepinephrine, serotonin, medial prefrontal cortex, hypothalamus

INTRODUCTION

Early studies suggested that endotoxin (lipopolysaccharide, LPS) stimulated the cerebral metabolism of norepinephrine (NE) and serotonin (5-HT). Pohorecky et al. (1972) showed that LPS injection increased the disappearance of radiolabeled NE and 5-HT administered to rats. Subsequent, neurochemical studies measuring the accumulation of catecholamine and serotonin metabolites have shown that LPS injection causes acute increases in cerebral NE catabolites (Heyes et al., 1989; Dunn, 1992a). These neurochemical changes were greatest in the hypothalamus; changes in other brain regions were significantly smaller. Dopamine (DA) metabolism was affected to a lesser extent (Dunn, 1992a). Increases in the serotonin catabolite, 5-hydroxyindolacetic acid (5-HIAA), were observed acutely following LPS, accompanied by increases in tryptophan (Heyes et al., 1989; Dunn, 1992a).

Unfortunately, such changes in postmortem neurochemical measurements do not necessarily reflect synaptic release. Therefore, in the present study, we used in vivo microdialysis to monitor extracellular concentrations of catecholamines in the hypothalamus and the prefrontal cortex of freely moving rats. We also tested the ability of indomethacin to prevent the effects of LPS on cerebral neurotransmitters, because the cyclo-oxygenase inhibitors have been described as blocking many of the physiological effects of LPS (Martich et al., 1992; Johnson et al., 1993).

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (250–300 g) were obtained from Harlan Sprague-Dawley Inc., Indianapolis, IN. The animals were housed individually under

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standard laboratory conditions (20–22°C, 50–55% humidity) and on a 12:12 hr light/dark cycle starting at 7:00 A.M. Water and Purina rat chow were available ad libitum.

Materials

Lipopolysaccharide from *Escherichia coli*, serotype 026:B6 prepared by trichloroacetic acid extraction, was purchased as a lyophilized powder from Sigma Chemical Company (St. Louis, MO, catalog number L-3755). All other chemicals were of analytical grade.

Microdialysis Probes

A concentric type of microdialysis probe was used (Kendrick, 1989). The inner silica tube was inserted into a 26-ga stainless steel tube (outer cannula). Polyethylene tubing (PE-20) was used for both inlet and outlet connections. The body holder was made from the tip of a tuberculin syringe filled with epoxy cement to hold the tubing. The dialysis membrane (Spectra Por Fiber, molecular weight cutoff 5–6,000) was attached to the end of stainless steel tube and sealed at the tip with epoxy cement. The active length of the dialysis membrane was 3.0 mm. For details see Lavicky and Dunn (1993).

Surgical Procedure

Animals were anesthetized using sodium pentobarbital (Nembutal 55 mg/kg ip, Abbott Labs, North Chicago, IL). Rats were placed in a stereotaxic apparatus, and guide cannulae for the microdialysis probe (the upper part of a 20-ga Becton Dickinson injection needle) were implanted and fixed using Cranioplastic cement (Plastics One, Roanoke, VA). Stereotaxic coordinates were taken from Pellegrino et al. (1979) with the nose bar set at +5.0 mm. The coordinates for the microdialysis probe guide cannula in medial hypothalamus were: A–P: +0.2 mm; L: 2.3 mm; V: immediately below dura, tilted medially at an angle of 10°, and in medial prefrontal cortex: A–P: +3.6 mm; L: 1.5 mm; V: immediately below dura, tilted medially at an angle of 15°. For detailed depiction of the placements, see Lavicky and Dunn (1993). These procedures were approved by the Louisiana State University Medical Center Animal Care Committee and conform to National Institutes of Health guidelines.

Microdialysis System

The inflow to the microdialysis probe was driven by a CMA/100 Microinjection Pump, and the outflow was collected by a CMA/140 Microfraction Collector, (BAS/Carnegie Medicine, West Lafayette, IN); both were connected through FEP inlet/outlet tubing (BAS). The perfusion fluid was artificial cerebrospinal fluid (aCSF) made according to Sharp et al. (1989): 2.3 mM $CaCl_2$, 1.2 mM Na_2HPO_4, 0.3 mM NaH_2PO_4, 3.4 mM KCl, 140 mM NaCl, pH 7.2. Samples were collected for 20 min at a flow rate of 2.25 μl/min directly into vials containing 800 pg of N-methyldopamine (NMDA: internal standard) in 50 μl of 0.1 M $HClO_4$ and 0.1 mM disodium ethylenediaminetetraacetic acid (EDTA). The samples were frozen soon after collection and stored at −70°C until analyzed.

HPLC

HPLC with electrochemical detection was used to determine the contents of NE, epinephrine, 3,4-dihydroxyphenylethyleneglycol (DHPG), 3-methoxy,4-hydroxyphenylethyleneglycol (MHPG), normetanephrine (NM), DA, 3-methoxytyramine (3-MT), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), and NMDA, with reference to freshly diluted standards (Sigma Chemical Co.). The system consisted of a Waters M-6000A Pump, and a WISP 712 refrigerated autoinjector (Waters Associates Inc., Milford, MA), a Spherisorb ODS 1, 25-cm, 5-micron reverse-phase column (Keystone Scientific, Inc., Bellefonte, PA), an LC-4B electrochemical detector with LC-17A glassy carbon electrode and LC-22A temperature controller (BAS, West Lafayette, IN). The working electrode was set at 0.78 V with respect to an Ag/AgCl reference electrode. The mobile phase contained 0.1 M NaH_2PO_4, pH 3.00, 0.10 mM EDTA, 0.5 mM octanesulfonic acid, and 4% acetonitrile. The flow rate was maintained at 1.0 ml/min. Minor changes of the pH, octanesulfonic acid concentration, and column temperature were made to obtain optimal separations.

Validation of Microdialysis Procedures

Relative in vitro recovery of microdialysis probes was determined as described by Lindefors et al. (1989). There was low variability in the recoveries, which were between 10–17% depending on the compound (see Lavicky and Dunn, 1993), in good agreement with values in the literature. Because the diffusion of materials from within brain tissue is likely to be different from that in a saline solution (Benveniste, 1989), we did not correct our in vivo data for these in vitro recoveries.

Procedures

Animals were fixed by Velcro to the spring-holder connected to the swivel (Ledger Technica Service, Kalamazoo, MI) and placed in a small cage (~20 × 22 cm). This familiarization procedure without microdialysis probe implantation, lasting the whole day, was performed for each animal 3 days prior to the experiment. On the experimental day, a microdialysis probe was inserted in the guide cannula of the rat 1 hr before sample

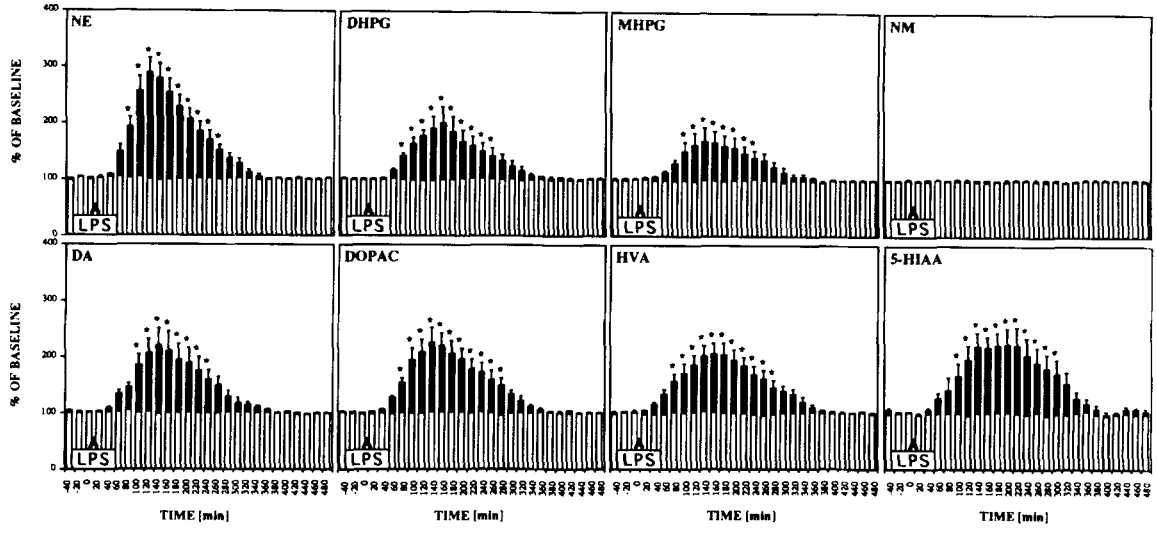

Fig. 1. The effect of LPS on NE and catabolites (DHPG, MHPG, and NM), and DA and catabolites (DOPAC and HVA), and of 5-HIAA in microdialysates from the medial hypothalamus. Rats were injected ip with 5 μg LPS or isotonic pyrogen-free saline, 3 hr after insertion of the dialysis probe. Each rat (n = 6) was retested 1 week later with the other injectate. Abscissa: time in minutes, starting 2 hr after insertion of the probe. Data are presented as the mean ± SEM of the percentage change from baseline. Solid bars, LPS; open bars, saline. *Significantly different from baseline ($P<0.05$, ANOVA followed by Student's paired t-test).

collection. Six 20-min samples were then collected, the last three of which were used to define the baseline. LPS (5 or 100 μg) or apyrogenic saline was injected ip. One week later the experiment was performed on the same group of animals (n = 6) with the treatments reversed. The same experiment was repeated on a separate group of animals using the high dose of LPS (100 μg, n = 6), but with two injections of indomethacin (10 mg/kg IP) or saline 1 hr before and after LPS treatment.

Histology

At the end of each experiment, methylene blue was injected through a dialysis probe without a membrane. The location of the dialysis probe was verified histologically. The data from animals with incorrect dialysis probe placements were excluded.

Statistics

All results are expressed as mean ± standard error of the mean (SEM). ANOVA was used to determine main effect of treatment. Statistical significance for the effects of the two treatments in the same animal was determined by paired Student's t-tests. Statistical significance was accepted for $P < 0.05$.

RESULTS

Effect of LPS on Medial Hypothalamus Dialysates

The response in the medial hypothalamus to the ip injection of 5 μg LPS is shown in Figure 1. We found statistically significant increases of NE (maximum 187%), DHPG (100%), MHPG (69%), DA (119%), DOPAC (118%), HVA (104%), and 5-HIAA (123%). No changes were observed in NM. The NE peak occurred about 2 hr after LPS administration, followed closely by DHPG and MHPG. DA (and DOPAC and HVA) showed a similar response, but was delayed slightly compared to NE. 5-HIAA reached a flat peak from about 2–4 hr following LPS. Concentrations of all metabolites reached baseline values which were achieved within 6 hr. Injection of apyrogenic saline did not alter baseline values of NT and their metabolites.

Effect of LPS on Medial Prefrontal Cortex Dialysates

The response in the prefrontal medial cortex to the ip injection of 5 μg LPS is shown in Figure 2. Statistically significant increases of NE (maximum 17%), DHPG (12%), MHPG (20%), DA (20%), DOPAC (19%), HVA (18%), and 5-HIAA (19%) were observed. The NE peak occurred about 120 min after LPS administration, followed by DHPG and MHPG, both at about 160 min. Statistically significant elevations of DA occurred around the same time. DOPAC, HVA, and 5-HIAA peaks closely resembled those in DA. Injection of apyrogenic saline did not alter baseline values of NT and their metabolites.

The response to a higher dose of LPS (100 μg) was similar but more pronounced (Fig. 3), and more closely

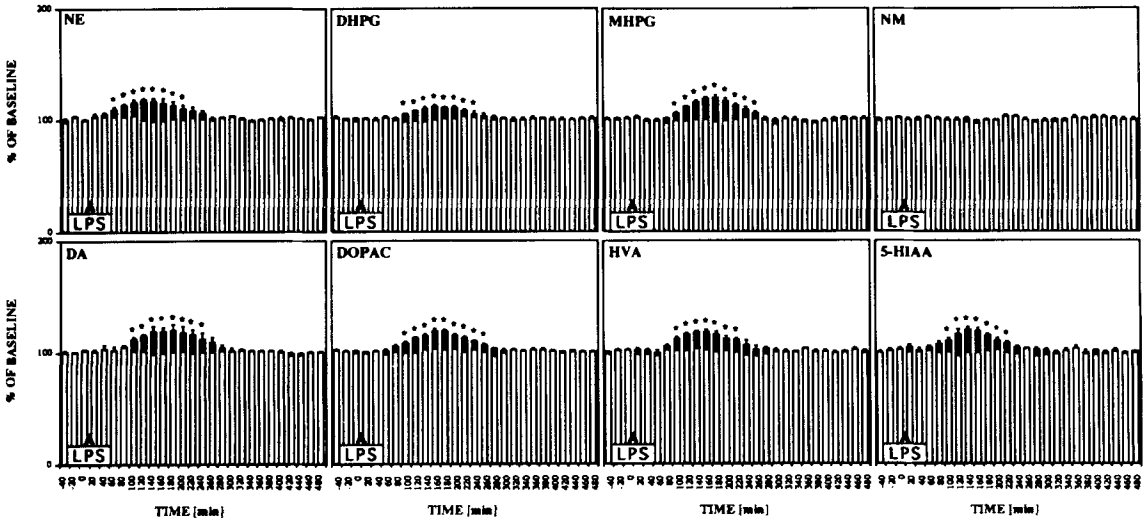

Fig. 2. The effect of LPS on NE and catabolites (DHPG, MHPG, and NM), and DA and catabolites (DOPAC and HVA), and of 5-HIAA in microdialysates from the medial prefrontal cortex. Rats were injected ip with 5 μg LPS or isotonic pyrogen-free saline, 3 hr after insertion of the dialysis probe. The experiment was conducted just like the one in Figure 1, except that microdialysis probes were inserted in the medial prefrontal cortex. *Significantly different from baseline ($P<0.05$, ANOVA followed by Student's paired t-test).

resembled the hypothalamic response to 5 μg. Not only were peak responses in metabolite concentrations larger, but the effects were prolonged compared to the lower dose. Whereas NE was significantly elevated after only 60 min, the peak (219% increase) occurred at 180 min, and did not return to baseline for nearly 6 hr. Increases in DHPG (124%), MHPG (127%), DA (157%), DOPAC (177%), and HVA (148%) were delayed slightly compared to those in NE, but returned to baseline at a similar time. However, the increase in 5-HIAA (206%) was not statistically significant until almost 3 hr, peaked between 5–6 hr, and was still significantly elevated after 7 hr. We did not observe any changes in dialysate NM with either dose of LPS.

Effect of Indomethacin Pretreatment

To determine the potential involvement of cyclooxygenase in the response to LPS, rats were pretreated with indomethacin (10 mg/kg ip) 1 hr before, and again 1 hr after LPS. This pretreatment completely prevented responses; no changes whatsoever were observed in the microdialysate concentrations of catecholamines, indolamines, or their catabolites (Fig. 4).

DISCUSSION

The present results indicate that LPS treatment elevates in vivo dialysate concentrations of DA and NE and their catabolites, and of 5-HIAA in both medial prefrontal cortex and medial hypothalamus. These changes suggest that release of both catecholamines (DA and NE), and probably also of 5-HT, were increased in both regions. This result is consistent with the data from postmortem neurochemical analyses of catabolites (Heyes et al., 1989; Masana et al., 1990; Mefford and Heyes, 1990; Dunn, 1992a). In the present microdialysis study, the peak response in NE concentration was the earliest at around 2–3 hr. This is comparable to that found in neurochemical studies of catabolites (Dunn, 1992a). The peak concentrations of DA and of catabolites of both catecholamines were a little later. However, peak concentrations of 5-HIAA were considerably later at around 4–6 hr. This delayed response in serotonin metabolism is also consistent with postmortem neurochemical studies (Dunn, 1992a).

The far greater sensitivity of the hypothalamus to the LPS treatment, as compared to that of the prefrontal cortex, is also consistent with the neurochemical data which showed larger increases in MHPG in this region compared to the other brain regions (Masana et al., 1990; Dunn, 1992a). This suggests that the dopaminergic, noradrenergic, and serotonergic projections to the hypothalamus are selectively activated by LPS, relative to the cortical projections. Because the cortical noradrenergic projections arise exclusively from the locus coeruleus, whereas the hypothalamus receives greater input from

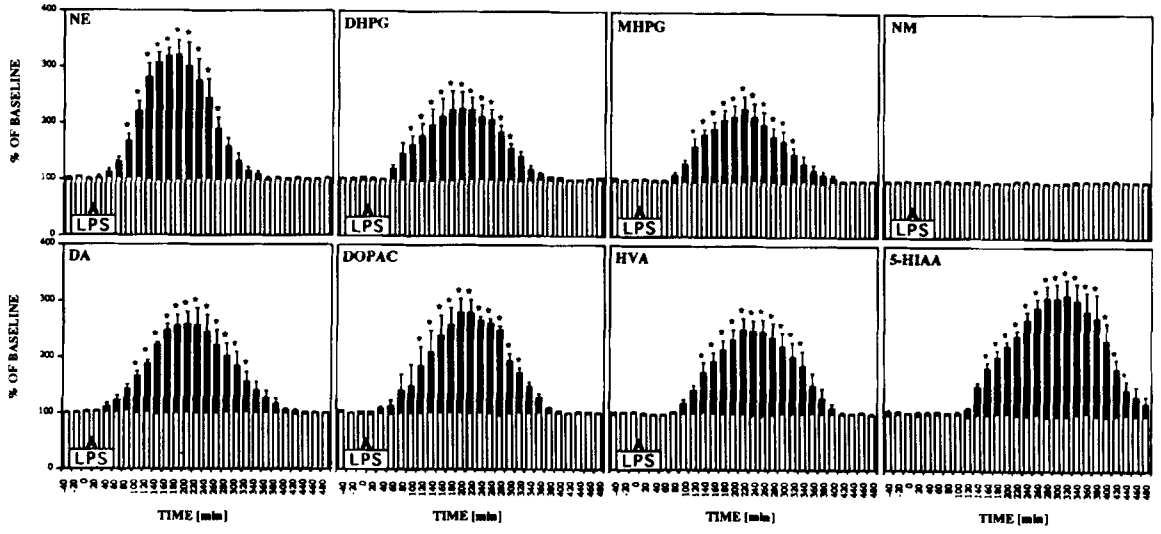

Fig. 3. The effect of LPS on NE and catabolites (DHPG, MHPG, and NM), and DA and catabolites (DOPAC and HVA), and of 5-HIAA in microdialysates from the medial prefrontal cortex. Rats were injected ip with 100 μg LPS or isotonic pyrogen-free saline, 3 hr after insertion of the dialysis probe. The experiment was conducted just like the one in Figure 2, except that a higher dose of LPS was used. *Significantly different from baseline ($P < 0.05$, ANOVA followed by Student's paired t-test).

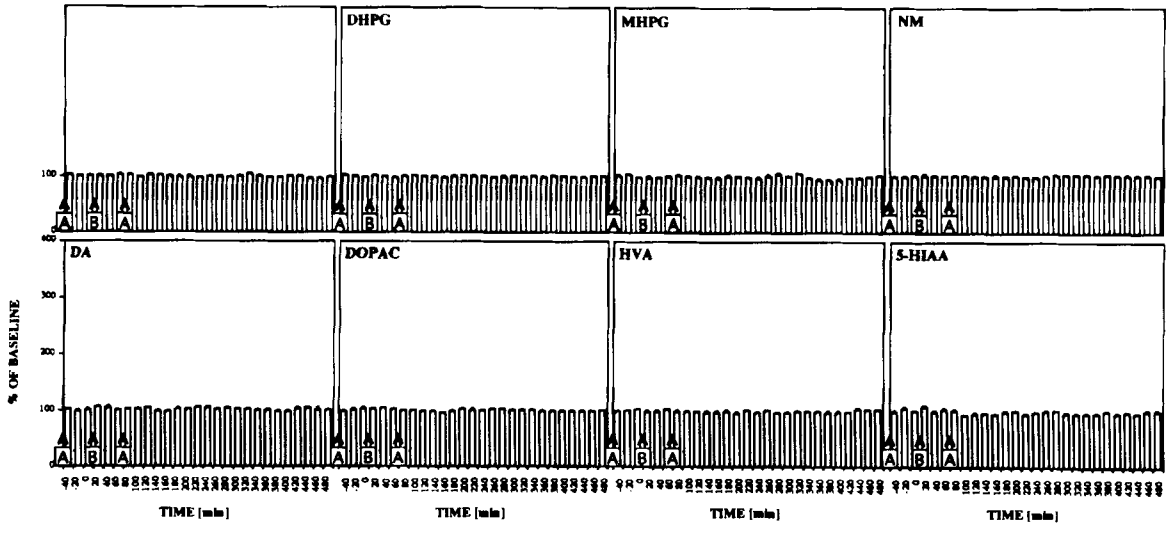

Fig. 4. The effect of LPS on NE and catabolites (DHPG, MHPG, and NM), and DA and catabolites (DOPAC and HVA), and of 5-HIAA in microdialysates from the medial prefrontal cortex of indomethacin-pretreated rats. Rats were injected ip with indomethacin (10 mg/kg) 2 and 4 hr (A), and with LPS (100 μg ip) or isotonic pyrogen-free saline 3 hr (B) after insertion of the dialysis probe. The experiment was conducted just like the one in Figure 3, except that rats were treated with indomethacin before and after LPS (or saline) injection.

other brain stem nuclei (A1, A2), these results suggest that the brainstem nuclei are differentially activated by LPS. The dopaminergic and serotonergic projections to cortex and hypothalamus also differ in the origins, so similar considerations may apply.

The coactivation of cerebral and serotonergic sys-

tems by LPS partially resembles that of physical or behavioral stressors like electric footshock or restraint (Stone, 1975; Dunn, 1988). The major difference is the smaller response to LPS in prefrontal cortex DA, and the selective effect of LPS on hypothalamic noradrenergic systems. The significance of these stress-related activations of cerebral catecholamines and serotonin has still to be elucidated, but they presumably play a role in the cognitive adaptation to stress.

The effect of pretreatment with cyclo-oxygenase inhibitors, such as indomethacin, on the cerebral responses to LPS has been varied. Most studies have found cyclo-oxygenase inhibitors to prevent the pituitary-adrenal responses (i.e., increased plasma ACTH and corticosterone), but Dunn and Chuluyan (1992) found no effect of indomethacin on ACTH and corticosterone responses to LPS. Heyes' group found that indomethacin prevented the catecholaminergic responses to IL-1 and LPS in rats (Masana et al., 1990) and mice (Mefford and Heyes, 1990). However, although we found that indomethacin and other cyclo-oxygenase inhibitors attenuated the noradrenergic responses in mice to IL-1, this was not true for LPS (Dunn and Chuluyan, 1992). Moreover, we did not observe any attenuation by indomethacin of the indolaminergic response to LPS or IL-1 (i.e., the increases in tryptophan and 5-HIAA). The reason for the apparent discrepancy between the neurochemical studies and the current studies with in vivo microdialysis is not obvious, although the former studies were conducted in mice, and the microdialysis studies in rats.

The effect of LPS on cerebral biogenic amines need not be direct. It is unlikely that LPS penetrates the blood-brain barrier. Although LPS has been shown to break down the barrier (Eckman et al., 1958), this effect is slow and starts only 3 hr after peripheral administration (Wispelwey et al., 1988). A potential mediator is interleukin-1 (IL-1). LPS administration is known to induce the peripheral production of IL-1 (Zuckerman et al., 1989), and in the brain following peripheral or central injections (Quan et al., 1994). However, when LPS was injected ip, IL-1β was found in the plasma, but not in the brain at 2 hr; it did not appear in the latter until 6 hr (Quan et al., 1994). Moreover, the IL-1-receptor antagonist (IL-1ra) was unable to prevent neurochemical responses to LPS, although it was effective against IL-1 (Dunn, 1992b). Thus, the mechanism of the neurochemical effects of LPS is not clear, although it apparently involves cyclo-oxygenase activation. Cyclo-oxygenase inhibitors can prevent cardiovascular responses to LPS (Martich et al., 1992), so that the cerebral changes in biogenic amines could be secondary to peripheral cardiovascular changes.

The present results using in vivo microdialysis provide further support for the suggestion that LPS administration stimulates the release of cerebral catecholamines, and possibly also of serotonin. The effect of LPS is regionally selective, such that the hypothalamus shows greater responses than the prefrontal cortex. Pretreatment with the cyclo-oxygenase inhibitor, indomethacin, prevented all the neurochemical responses observed with LPS.

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REFERENCES

Benveniste H (1989): Brain microdialysis. J Neurochem 52:1667–1679.

Dunn AJ (1988): Stress-related changes in cerebral catecholamine and indoleamine metabolism: Lack of effect of adrenalectomy and corticosterone. J Neurochem 51:406–412.

Dunn AJ (1992a): Endotoxin-induced activation of cerebral catecholamine and serotonin metabolism: Comparison with interleukin-1. J Pharmacol Exp Ther 261:964–969.

Dunn AJ (1992b): The role of interleukin-1 and tumor necrosis factor α in the neurochemical and neuroendocrine responses to endotoxin. Brain Res Bull 29:807–812.

Dunn AJ, Chuluyan H (1992): The role of cyclo-oxygenase and lipoxygenase in the interleukin-1-induced activation of the HPA axis: Dependence on the route of injection. Life Sci 51:219–225.

Eckman PL, King WM, Brunson JG (1958): Studies on the blood brain barrier. I. Effects produced by a single injection of the gram-negative endotoxin the permeability of the cerebral vessels. Am J Pathol 34:631–639.

Heyes MP, Quearry BJ, Markey SP (1989): Systemic endotoxin increases L-tryptophan, 5-hydroxyindoleacetic acid, 3-hydroxykynurenine and quinolinic acid content of mouse cerebral cortex. Brain Res 491:173–179.

Johnson RW, Curtis SE, Dantzer R, Kelley KW (1993): Central and peripheral prostaglandins are involved in sickness behavior in birds. Physiol Behav 53:127–131.

Kendrick KM (1989): Use of microdialysis in neuroendocrinology. Methods Enzymol 168:182–205.

Lavicky J, Dunn AJ (1993): Corticotropin-releasing factor stimulates catecholamine release in hypothalamus and prefrontal cortex in freely moving rats as assessed by microdialysis. J Neurochem 60:602–612.

Lindefors N, Amberg G, Ungerstedt U (1989): Intracerebral microdialysis: I. Experimental studies of diffusion kinetics. J Pharmacol Methods 22:141–156.

Martich GD, Parker MM, Cunnion RE, Suffredini AF (1992): Effects of ibuprofen and pentoxifylline on the cardiovascular response of normal humans to endotoxin. J Appl Physiol 73:925–931.

Masana MI, Heyes MP, Mefford IN (1990): Indomethacin prevents increased catecholamine turnover in rat brain following systemic endotoxin challenge. Prog Neuropsychopharmacol Biol Psychiatry 14:609–621.

Mefford IN, Heyes MP (1990): Increased biogenic amine release in mouse hypothalamus following immunological challenge: Antagonism by indomethacin. J Neuroimmunol 27:55–61.

Pellegrino LJ, Pellegrino AS, Cushman AJ (1979): "Stereotaxic Atlas of the Rat Brain." New York: Plenum Press.

Pohorecky LA, Wurtman RJ, Taam D, Fine J (1972): Effects of endotoxin on monoamine metabolism in the rat. Proc Soc Exp Biol Med 140:739–746.

Quan N, Sundar QN, Weiss JM (1994): Induction of interleukin-1 in various brain regions after peripheral and central injections of lipopolysaccharide. J Neuroimmunol 49:125–134.

Sharp T, Bramwell SR, Clark D, Grahame-Smith DG (1989): In vivo measurement of extracellular 5-hydroxytryptamine in hippocampus of the anaesthetized rat using microdialysis: changes in relation to 5-hydroxytryptaminergic neuronal activity. J Neurochem 53:234–240.

Stone EA (1975): Stress and catecholamines. In Friedhoff AJ (ed): "Catecholamines and Behavior." New York: Plenum Press, pp 31–72.

Wispelwey B, Lesse AJ, Hansen EJ, Scheld WM (1988): *Haemophilus influenzae* lipopolysaccharide-induced blood brain barrier permeability during experimental meningitis in the rat. J Clin Invest 82:1339–1346.

Zuckerman SH, Shellhaas J, Butler LD (1989): Differential regulation of lipopolysaccharide-induced interleukin-1 and tumor necrosis factor synthesis: Effects of endogenous and exogenous glucocorticoids and the role of the pituitary-adrenal axis. Eur J Immunol 19:301–305.