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Lipopolysaccharide administration produces time-dependent and region-specific alterations in tryptophan and tyrosine hydroxylase activities in rat brain

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Summary. The present study examined the effect of systemic administration of lipopolysaccharide (LPS; 100 and 250 μg/kg, i.p.) on tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) activities in frontal cortex, striatum and midbrain of the rat. Enzyme activities were determined by measuring accumulation of the transient intermediates 5-hydroxytrptophan (5-HTP) and L-dihydroxyphenylalanine (L-DOPA) following in vivo administration of the decarboxylase inhibitor, NSD 1015. TPH activity was increased 2 hours after administration of LPS (100 and 250 μg/kg) in both frontal cortex and midbrain, and a secondary increase was seen in the midbrain 12 hours after challenge. LPS provoked an increase in TH activity in the midbrain only, and this was evident for up to 24 hours after LPS administration. Thus in addition to previous studies demonstrating that LPS increases in vivo NA, DA and 5-HT release, this study shows that LPS increases the activity of the rate-limiting enzymes responsible for their synthesis.

Keywords: Lipopolysaccharide, cytokine, tyrosine hydroxylase, tryptophan hydroxylase, serotonin, catecholamine.

Introduction

Systemic administration of the pro-inflammatory cytokine inducer, lipopolysaccharide (LPS), is used as an animal model of acute infection or inflammation (Tilders et al., 1994; Givalois et al., 1994). It has been postulated that an LPS challenge is interpreted by the brain as a stressor, causing changes in central neurotransmitter release (Anisman et al., 1993). In this regard, several studies have reported central serotonergic, dopaminergic and noradrenergic alterations following systemic LPS administration, by measuring the concentration of the parent amines and their respective metabolites, using both in vivo microdialysis (Connor et al., 1999; Borowski et al., 1998; Lavicky

and Dunn, 1995; Linthorst et al., 1995) and post mortem tissue analysis (MohanKumar et al., 1999; Dunn, 1992). However, the impact of LPS on the activity of tryptophan hydroxylase (TPH) and tyrosine hydroxylase (TH), the initial and rate limiting enzymes in the synthesis of serotonin (5-HT) and dopamine (DA)/noradrenaline (NA) respectively, has not been investigated.

Previous animal studies have reported altered TH and TPH activities in the cell body and terminal regions of serotonergic, dopaminergic and noradrenergic neurons, in response to stressful stimuli. For example, it has been demonstrated that long-term isolation stress increased TH activity in midbrain and forebrain structures (Toru, 1982), while acute or repeated auditory stress induced activation of TPH observed in cortical and midbrain regions (Singh et al., 1994, 1990; Boadle-Biber et al., 1989). Thus it is possible that exposure to an LPS challenge which provokes "stress-like" changes in monoamine neurotransmitter activity will increase the activity of these synthetic enzymes.

In the present study, we examined the effect of systemic administration of LPS, on the activity of TPH and TH in a cell body region (midbrain), and terminal regions (frontal cortex and striatum) of the rat. The activity of TPH was assessed by measuring the accumulation of 5-hydroxytryptophan (5-HTP), and the activity of TH by measuring L-dihydroxyphenylalanine (L-DOPA) accumulation, after in vivo administration of m-hydroxybenzylhydrazine dihydrochloride (NSD 1015), an aromatic amino acid decarboxylase inhibitor. Both 5-HTP and L-DOPA usually occur as transient intermediates and are not normally detected in brain tissue, but administration of NSD 1015 prevents the decarboxylation step common to both compounds and thus allows them to accumulate in vivo (Carlsson et al., 1972). As LPS-induced effects may demonstrate differential times of onset, as well as differential response duration, TH and TPH activities were examined at different timepoints (up to 24 hr) following acute LPS administration.

Materials and methods

Subjects

Adult male Sprague Dawley rats weighing between 250 and 350 grams were obtained from Biological Laboratories, Ballina, Ireland. The animals were housed four per cage in a temperature (22 ± 2°C), humidity (relative humidity 60–80%), and light controlled (12 hr light: 12 hr dark; lights on: 08.00 hr/lights off: 20.00 hr) environment. Food and water were available ad libitum.

The experimental protocol was carried out under the guidelines of the Animal Welfare Committee, National University of Ireland, Galway, Ireland, and were in compliance with the European Communities Council directive (86/609/EEC).

Drug treatments

Escherichia coli LPS, serotype 0111:B4 (Sigma Chemical Co., Poole, UK) was dissolved in sterile 0.9% NaCl at a concentration of either 100 μg/ml and 250 μg/ml and administered at an injection volume of 1 ml/kg by the intraperitoneal route. Control animals received an equal volume of 0.9% NaCl alone as a vehicle.

m-Hydroxybenzylhydrazine dihydrochloride (NSD 1015; Sigma Chemical Co., Poole, UK) was dissolved in sterile 0.9% NaCl at a concentration of 100 mg/ml and was administered at an injection volume of 1 ml/kg by the intraperitoneal route.

Experimental procedure

Drug administration: Animals were injected with NSD 1015 (100 mg/kg, i.p.) either 2, 6, 12, or 24 hours following LPS challenge. Animals were killed 1 hour following administration of NSD 1015. The selection of this dose and time were determined based on data generated in our laboratory indicating that NSD 1015 (100 mg/kg; i.p.) produces optimal increases in 5-HTP and L-DOPA concentrations in rat brain 1 hour after administration without altering behaviour and apparent well-being of the rat.

Brain dissection and preparation of tissue: One hour following NSD 1015 injection, rats were killed by decapitation and the brains were rapidly removed from the skull. The frontal cortex, striatum, and midbrain of each rat was dissected on an ice cold plate (Popov et al., 1967). Each piece of tissue was sonicated (MSE sonicator) in 1.0 ml of homogenizing solution (4°C) comprised of 0.1 M citric acid (Merck, Darmstadt, Germany), 0.1 M sodium dihydrogen phosphate (Merck, Darmstadt, Germany), 1.4 mM 1-octane sulfonic acid (Sigma Chemical Co., Poole, UK), 0.1 mM EDTA (Sigma Chemical Co., Poole, UK) and 10% (v/v) methanol 215 HPLC grade (Romil Ltd., Cambridge, UK). This solution (mobile phase) was adjusted to pH 2.8 using 4 N NaOH (BDH Chemicals Ltd., Poole, UK) and spiked with 20 ng/50 µl of N-methyl dopamine (Sigma Chemical Co., Poole, UK) as an internal standard. The homogenates were centrifuged (Hettich 1306, Mikro-Rapid K Bench Model) at 12,000 rpm at 4°C for 15 minutes and stored at −20°C until analysis was carried out.

HPLC analysis of L-DOPA and 5-HTP concentrations

Concentrations of 5-HTP and L-DOPA in both samples and standards were measured by high performance liquid chromatography (HPLC) using an automated HPLC system (Shimadzu) coupled with electrochemical detection (Seyfried et al., 1986). Samples stored at −20°C were allowed to thaw at room temperature and centrifuged at 12,000 RPM at 4°C for 15 minutes. 150 µl aliquot of the supernatant was dispensed into the assay vial used for HPLC measurements. A 20 µl sample of the supernatant was injected directly onto a reverse phase column (Licrosorb RP-18, 250 × 4.00 mm internal diameter, particle size 5 mm) for separation of 5-HTP and L-DOPA (flow rate 1 ml/min mobile phase, pressure 200 bar, 30°C). An electrochemical detector (Shimadzu) was coupled to the HPLC system and was set at a potential of +0.8 V. 5-HTP and L-DOPA were quantified using a Merck-Hitachi D-2000 integrator and concentrations were expressed as ng per g fresh weight of brain tissue.

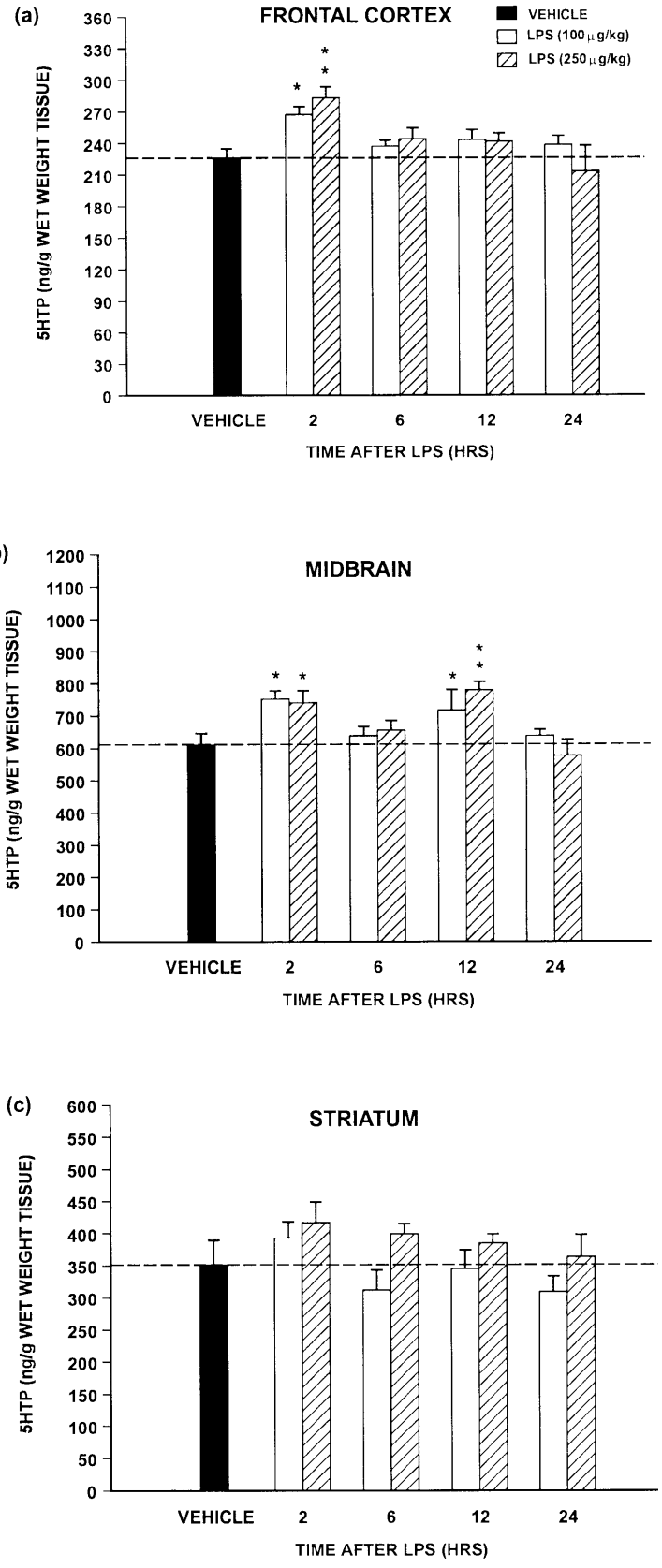
Statistical analysis

Initially, a one-way analysis of variance (ANOVA) was performed on all data and if statistically significant changes were found, the data was further analysed using post hoc comparisons with Fisher's LSD multiple range test. Data was deemed significant when $P < 0.05$. Results are expressed as group means with standard error of the mean (SEM).

Results

Tryptophan hydroxylase activity

There was a significant effect of LPS challenge on cortical [$F(8, 51) = 3.082$, $P = 0.0078$] and midbrain [$F(8, 52) = 3.40$, $P = 0.0027$] concentrations of 5-HTP. Post hoc analysis demonstrated that there was a significant increase in cortical 5-HTP due to 100 ($P < 0.05$) and 250 µg/kg LPS ($P < 0.01$) 2 hours after challenge (Fig. 1a). Midbrain 5-HTP concentrations were increased following administration of 100 µg/kg LPS, 2 and 12 hours after challenge ($P < 0.05$) and also following 250 µg/kg LPS, 2 hours ($P < 0.05$) and 12 hours


(a)
FRONTAL CORTEX
VEHICLE
LPS (100 μg/kg)
LPS (250 μg/kg)
5HTP (ng/g WET WEIGHT TISSUE)
360
330
300
270
240
210
180
150
120
90
60
30
0
VEHICLE
2
6
12
24
TIME AFTER LPS (HRS)
(b)
MIDBRAIN
5HTP (ng/g WET WEIGHT TISSUE)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
VEHICLE
2
6
12
24
TIME AFTER LPS (HRS)
(c)
STRIATUM
5HTP (ng/g WET WEIGHT TISSUE)
600
550
500
450
400
350
300
250
200
150
100
50
0
VEHICLE
2
6
12
24
TIME AFTER LPS (HRS)

($P < 0.01$) after challenge (Fig. 1b). ANOVA revealed that there was no significant effect of LPS challenge on 5-HTP concentration in the striatum (Fig. 1c).

Tyrosine hydroxylase activity

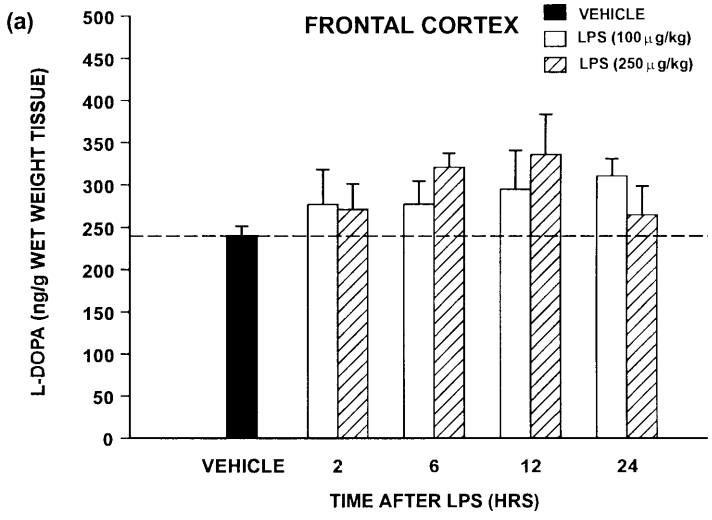
ANOVA revealed that there was no significant effect of LPS challenge on L-DOPA concentration in the frontal cortex (Fig. 2a). There was a significant effect of LPS on L-DOPA concentration [$F(8, 52) = 4.14$, $P = 0.0009$] in the midbrain. Post hoc analysis revealed a significant increase in L-DOPA 6 hours ($P < 0.05$) and 24 hours ($P < 0.01$) after 100 μg/kg LPS, and a significant increase in L-DOPA 2 and 12 hours after both doses of LPS ($P < 0.01$) (Fig. 2b). ANOVA revealed that there was no significant effect of LPS challenge on L-DOPA concentration in the striatum (Fig. 2c).

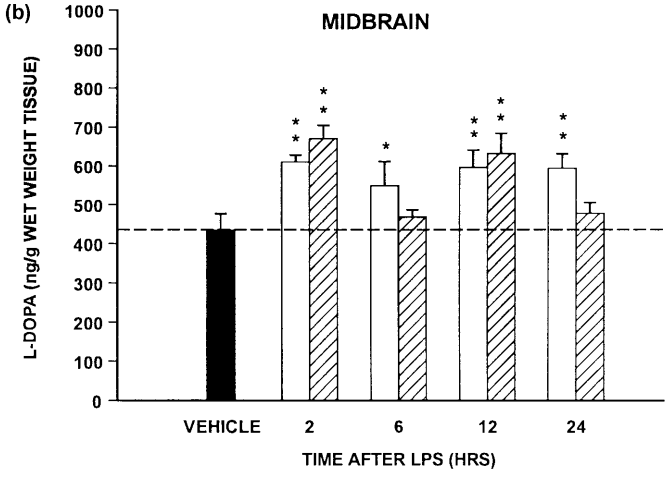
Discussion

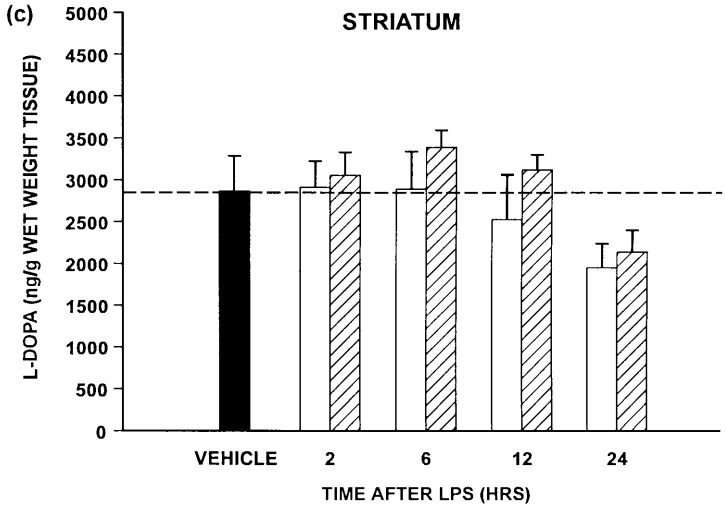
The results of the present study indicate that both of the hydroxylase enzymes, TPH and TH are activated in response to peripheral LPS administration in a time-dependent and region-specific manner. Both doses of LPS used produced similar effects, thus suggesting that LPS increases hydroxylase activity regardless of concentration.

Previous studies examining the effects of immunological activation on the serotonergic system have demonstrated that systemic treatment with LPS increases serotonergic neurotransmission in rat hippocampus, hypothalamus, nucleus accumbens, frontal cortex and brain stem (Connor et al., 1999; MohanKumar et al., 1999; Molina-Holgado and Guaza, 1996; Linthorst et al., 1996; Lavicky and Dunn, 1995; Dunn, 1992). Neurons containing 5-HT are clustered around the raphe area of the midbrain, and cells of the dorsal and median raphe nuclei innervate limbic structures such as the hippocampus, and cortex as well as thalamus and hypothalamus (Deutch and Roth, 1999). In the present study increased 5-HTP accumulation occurred in both the frontal cortex and midbrain 2 hours following LPS administration, and also 12 hours following LPS in the midbrain. In the frontal cortex, the increase in TPH activity that occurred 2 hours after challenge was transient, with 5-HTP concentrations having returned to baseline values when measured again 6 hours following LPS administration. In contrast TPH activity in the midbrain responded to LPS in a biphasic manner, with significant increases at both 2 and 12 hours following treatment. Whilst there was no significant effect of LPS on 5-HTP accumulation in the striatum, a modest increase in the striatal 5-HTP concentration was evident 2 hr following both doses of LPS, this may well indicate that striatal TPH activity was increased following LPS administration but that the striatum is less sensitive than the cortex in this

Fig. 1. Effect of acute LPS administration on 5-HTP concentrations in **a** frontal cortex, **b** midbrain and **c** striatum of the rat. Data expressed as means with standard errors (n = 5–6). * $P < 0.05$, ** $P < 0.01$ vs. vehicle treated controls (Fisher's LSD)


(a)
FRONTAL CORTEX
VEHICLE
LPS (100 μg/kg)
LPS (250 μg/kg)
L-DOPA (ng/g WET WEIGHT TISSUE)
500
450
400
350
300
250
200
150
100
50
0
VEHICLE
2
6
12
24
TIME AFTER LPS (HRS)


(b)
MIDBRAIN
L-DOPA (ng/g WET WEIGHT TISSUE)
1000
900
800
700
600
500
400
300
200
100
0
VEHICLE
2
6
12
24
TIME AFTER LPS (HRS)


(c)
STRIATUM
L-DOPA (ng/g WET WEIGHT TISSUE)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
VEHICLE
2
6
12
24
TIME AFTER LPS (HRS)

regard. Thus increased TPH activity was evident in both cell body and nerve terminal regions following acute LPS administration. These results are somewhat similar to those observed following exposure to an acute auditory stressor, in that TPH activity was increased in both the cortex and midbrain following stressor exposure (Singh et al., 1994, 1990), thus emphasising a similarity between exposure to psychological stress and to an immunological/metabolic stressor such as an LPS challenge.

Previous studies using both post-mortem tissue analysis and in vivo microdialysis to examine concentrations of catecholamines and their metabolites in the cortex, hypothalamus and brain stem (Lavicky and Dunn, 1995; Dunn, 1992), have reported changes in catecholamine release in response to LPS. Dopamine and noradrenaline cell bodies are present in the midbrain, locus corelus, and ventral tegmental area, respectively, and these neurons give rise to extensive forebrain catecholamine innervations including those in the striatum and several cortical regions (Deutch and Roth, 1999). In the present study the only brain region that exhibited a significant increase in L-DOPA accumulation as a result of LPS administration was the midbrain, indicating that LPS provoked a significant increase in TH activity in catecholamine cell body regions. The increase in midbrain TH activity induced by LPS persisted for up to 24 hr following administration and did not appear to be dose dependent, if anything the lower dose of LPS (100 μg/kg) gave the most consistent increases in midbrain TH activity over time.

It is likely that the observed increase in TH and TPH activities are secondary to neurotransmitter release induced by LPS, and that the rate-limiting enzymes are activated in order to replenish the neurotransmitter stores following neurotransmitter release. It has been previously demonstrated in the case of TH, that stimulation of transmitter release necessitates vesicle refilling, thus depleting the TH-accessible pool, reducing end-product inhibition by catecholamines, and resulting in an enhancement of TH activity (Masserano et al., 1989). Thus it is surprising that in the present study significant LPS-induced changes in TH activity were confined to the cell body region (midbrain).

Previous studies that have examined the effect of LPS on neurotransmitter activity demonstrate that the LPS-induced increase in catecholamine release occurs earlier and is shorter lived than the LPS-induced increase in serotonergic activity (Lavicky and Dunn, 1995; Dunn, 1992). Thus it is puzzling that in the present study LPS-induced increases in TH activity occurred at the same time as, and were evident for, a longer duration than LPS-induced increases in TPH activity.

It is possible that pro-inflammatory cytokines that are synthesised within the brain in response to systemic LPS administration (Pitossi et al., 1997; Jansky et al., 1995; Hillhouse and Mosley, 1993) may act on neurotransmitter

Fig. 2. Effect of acute LPS administration on L-DOPA concentrations in **a** frontal cortex, **b** midbrain and **c** striatum of the rat. Data expressed as means with standard errors (n = 5–6). * $P < 0.05$, ** $P < 0.01$ vs. vehicle treated controls (Fisher's LSD)

circuits to alter neurotransmitter release, or to affect the enzymes involved in synthesis of these neurotransmitters. In this regard it was previously demonstrated that i.c.v. administration of the proinflammatory cytokine IL-1β increases TH activity in the median eminence 1 hour following administration in rats (Abreu et al., 1994), however the effect of IL-1β on TH was not assessed at any other timepoint following administration, or in any other brain region.

In summary, the results of the present study demonstrate that the hydroxylating enzymes TPH and TH are activated following acute LPS administration in rats in a time-dependent and region-specific manner. As to whether LPS itself or products induced by LPS such as IL-1β or other such proinflammatory cytokines stimulates the activation of these enzymes directly, or the increased enzyme activities occur simply as a mechanism to replenish neurotransmitters following release remains to be elucidated.

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References

Abreu P, Llorente E, Hernandez MM, Gonzalez MC (1994) Interleukin-1 beta stimulates tyrosine hydroxylase activity in the median eminence. Neuroreport 5: 1356–1358

Anisman H, Zalcman S, Zacharko RM (1993) The impact of stressors on immune and central neurotransmitter activity bidirectional communication. Rev Neurosci 4: 147–180

Boadle-Biber MC, Corley KC, Graves L, Phan TH, Rosecrans J (1989) Increase in the activity of tryptophan hydroxylase from cortex and midbrain of male Fischer 344 rats in response to acute or repeated sound stress. Brain Res 482: 306–316

Borowski T, Kokkinidis L, Merali Z, Anisman H (1998) Lipopolysaccharide, central in vivo biogenic amine variations, and anhedonia. Neuroreport 9: 3797–3802

Carlsson A, Davis JN, Kehr W, Lindqvist M, Atack CV (1972) Simultaneous measurement of tyrosine and tryptophan hydroxylase activities in brain in vivo using an inhibitor of the aromatic amino acid decarboxylase. Naunyn Schmiedebergs Arch Pharmacol 275: 153–168

Connor TJ, Song C, Leonard BE, Anisman H, Merali Z (1999) Stressor-induced alterations in serotonergic activity in an animal model of depression. Neuroreport 10: 523–528

Deutch AY, Roth RH (1999) Neurotransmitters. In: Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR (eds) Fundamental neuroscience. Academic Press, San Diego, pp 193–234

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

Givalois L, Dornand J, Mekaouche M, Solier MD, Bristow AF, Ixart G, Siaud P, Assenmacher I, Barbanel G (1994) Temporal cascade of plasma level surges in ACTH, corticosterone and cytokines in endotoxin-challenged rats. Am J Physiol 267: R164–R170

Hillhouse EW, Mosley K (1993) Peripheral endotoxin induces hypothalamic immunoreactive interleukin-1 in the rat. Br J Pharmacol 109: 289–290

Jansky L, Vybiral S, Pospisilova D, Roth J, Dornand J, Zeiberger E, Kaminkova J (1995) Production of systemic and hypothalamic cytokines during the early phase of endotoxin fever. Neuroendocrinology 62: 55–61

Lavicky J, Dunn AJ (1995) Endotoxin administration stimulates cerebral catecholamine release in freely moving rats as assessed by microdialysis. J Neurosci Res 40: 407–413
Linthorst ACE, Flachskamm C, Muller-Preuss P, Holsboer F, Reul JMHM (1995) Effect of bacterial endotoxin and interleukin-1 beta on hippocampal serotonergic neurotransmission, behavioral activity, and free corticosterone levels: an in vivo microdialysis study. J Neurosci 15: 2920–2934
Linthorst ACE, Flachskamm C, Holsboer F, Reul JMHM (1996) Activation of serotonergic and noradrenergic neurotransmission in the rat hippocampus after peripheral administration of bacterial endotoxin: involvement of the cyclo-oxygenase pathway. Neuroscience 72: 989–997
Masserano JM, Vuilliet PR, Tank AW, Weiner N (1989) The role of tyrosine hydroxylase in the regulation of catecholamine synthesis. In: Trendelenburg U, Weiner N (eds) Handbook of experimental pharmacology. Springer, Berlin Heidelberg New York Tokyo, pp 427–469
MohanKumar SM, MohanKumar PS, Quadri SK (1999) Lipopolysaccharide induced changes in monoamines in specific areas of the brain: blockade by interleukin-1 receptor antagonist. Brain Res 824: 232–237
Molina-Holgado F, Guaza C (1996) Endotoxin administration induced differential neurochemical activation of brain stem nuclei. Brain Res Bull 40: 151–156
Pitossi F, del Rey A, Kabiersch A, Besedovsky H (1997) Induction of cytokine transcripts in the central nervous system and pituitary following peripheral administration of endotoxin to mice. J Neurosci Res 48: 287–298
Popov N, Pohle W, Rosier V, Matthies H (1967) Regionale verteilung von gamma-aminobuttersaure, glutaminsaure, asparaginsaure, dopamin, noradreanlin and serotonin in rattenhirn. Acta Med Biol Germ 18: 695–702
Seyfried CA, Adam G, Greve T (1986) An automated direct-injection HPLC method for the electrochemical/fluorometric quantitation of monoamines and related compounds optimized for the screening of large numbers of animals. Biomed Chromatogr 1: 78–88
Singh VB, Onaivi ES, Phan TH, Boadle-Biber MC (1990) The increases in rat cortical and midbrain tryptophan hydroxylase activity in response to acute or repeated sound stress are blocked by bilateral lesions to the central nucleus of the amygdala. Brain Res 530: 49–53
Singh VB, Corley KC, Krieg RJ, Phan TH, Boadle-Biber MC (1994) Sound stress activation of tryptophan hydroxylase blocked by hypophysectomy and intercranial RU 38486. Eur J Pharmacol 256: 177–184
Tilders FJH, DeRijk RH, Van Dam A, Vincent VAM, Schotanus K, Persoons JHA (1994) Activation of the hypothalamic-pituitary-adrenal axis by bacterial endotoxins: Routes and interrmediate signals. Psychoneuroendocrinology 19: 209–232
Toru M (1982) Increased tyrosine hydroxylase activity in frontal cortex of rats after long-term isolation stress. Encephale 8: 315–317

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