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ORIGINAL ARTICLE

The initial fall in arterial pressure evoked by endotoxin is mediated by the ventrolateral periaqueductal gray

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SUMMARY

This study tested the hypothesis that the initial fall in arterial pressure evoked by lipopolysaccharide (LPS) is mediated by the ventrolateral column of the midbrain periaqueductal gray region (vlPAG). To test this hypothesis, the local anaesthetic lidocaine (2%; 0.1 μL, 0.2 μL or 1.0 μL), the delta opioid receptor antagonist naltrindole (2 nmol) or saline was microinjected into the vlPAG of isoflurane-anaesthetized rats bilaterally and LPS (1 mg/kg) or saline was administered intravenously 2 min later. Both lidocaine and naltrindole inhibited LPS-evoked hypotension significantly but did not affect arterial pressure in saline-treated control animals. Neither lidocaine nor naltrindole altered heart rate significantly in either LPS-treated or control animals. Microinjection of lidocaine or naltrindole into the dorsolateral PAG was ineffective. These data indicate that the vlPAG plays an important role in the initiation of endotoxic hypotension and further show that delta opioid receptors in the vlPAG participate in the response.

Key words: delta receptor, Endotoxin, endotoxin shock, lipopolysaccharide, opioid receptor, periaqueductal gray, preoptic area.

INTRODUCTION

Lipopolysaccharide (LPS) is commonly used to investigate the haemodynamic effects of Gram-negative endotoxemia. Although it is often assumed to lower arterial pressure by producing vasodilation through a direct action on the vasculature and/or by stimulating cytokine release[1] previous studies from our laboratory indicate that LPS initially lowers arterial pressure through a central mechanism.[2–4] Specifically, we found that LPS-evoked hypotension could be prevented by microinjecting the local anaesthetic lidocaine or the alpha-adrenergic receptor antagonist phentolamine into the hypothalamic preoptic area (POA) of conscious or anaesthetized rats.[2–4] Lipopolysaccharide-evoked hypotension could also be prevented by injecting lidocaine into the nucleus tractus solitarius or by transecting the vagus nerve suggesting that vagus nerve afferents convey the immune signal from circulating LPS to the brain.[3] These findings indicate that endotoxic hypotension is initiated through a central mechanism that involves activation of vagal afferents and stimulation of norepinephrine release in the POA. It remains to be determined how the POA initiates the precipitous fall in arterial pressure in response to LPS.

This study tested the hypothesis that the ventrolateral midbrain periaqueductal gray (vlPAG) region plays an important role in the initiation of endotoxic hypotension. The POA directly innervates the vlPAG as well as other cardioregulatory brain regions.[5] Electrical stimulation of, or excitatory amino acid injection into, the POA lowers arterial pressure[6–8] and this response can be prevented by injecting lidocaine or kainic acid into the vlPAG.[8,9] Destruction of neurons in the nucleus raphe magnus also effectively blocks the depressor response produced by POA stimulation,[9] consistent with evidence that vlPAG activation lowers arterial pressure by inhibiting sympathetic nerve activity through a descending pathway through the midline raphe nuclei and ventrolateral medulla.[10–12] Inactivation of the vlPAG also prevents the fall in arterial pressure caused by severe haemorrhage[13] and visceral nociception.[14] These findings indicate that the vlPAG is a critical component of a descending pathway that lowers arterial pressure as a consequence of trauma, visceral pain and severe blood loss.[7,10]

There is also evidence that delta opioid receptors in the vlPAG participate in these cardiovascular responses. Microinjection of delta, but not mu or kappa, opioid receptor agonists into the caudal vlPAG lowers arterial pressure[15] and, conversely, microinjection of a delta, but not mu or kappa, receptor antagonist into the vlPAG prevents the fall in arterial pressure caused by severe blood loss or visceral nociception.[14,16] If the same, or a similar, pathway initiates endotoxic hypotension then blockade of delta receptors in the vlPAG should also inhibit the effects of LPS. The present study thus evaluated whether microinjection of either lidocaine or the delta receptor antagonist naltrindole into the vlPAG inhibits the fall in arterial pressure evoked by LPS.

RESULTS

Lipopolysaccharide (1 mg/kg, intravenous (i.v.)) administration produced a biphasic fall in arterial pressure (Fig. 1a), as shown

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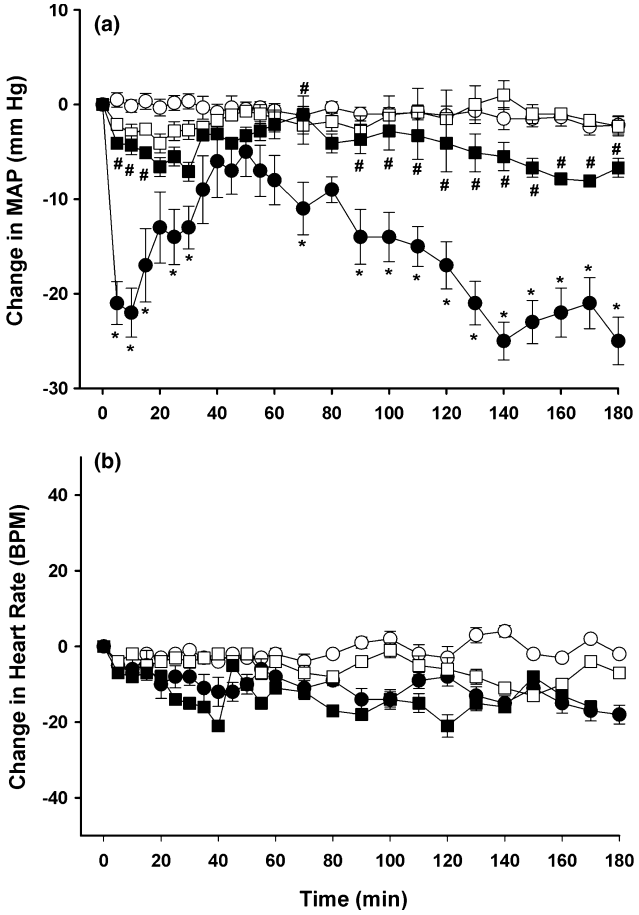

Fig. 1 Lidocaine injection into the ventrolateral midbrain periaqueductal gray region (vlPAG) prevents the fall in arterial pressure caused by lipopolysaccharide (LPS). Lidocaine (2%; 0.2 μL) or saline was injected bilaterally into the caudal vlPAG of isoflurane-anaesthetized rats and, 2 min later, LPS (1 mg/kg) or saline was administered intravenously (n = 6). Mean arterial pressure (MAP; Panel a) and heart rate (Panel b) were recorded for 180 min. $^{*}P < 0.05$, differs from saline + saline. $^{\#}P < 0.05$, differs from saline + LPS. (–○–) Saline + saline; (–●–) saline + LPS; (–□–) lidocaine + saline; (–■–) lidocaine + LPS.

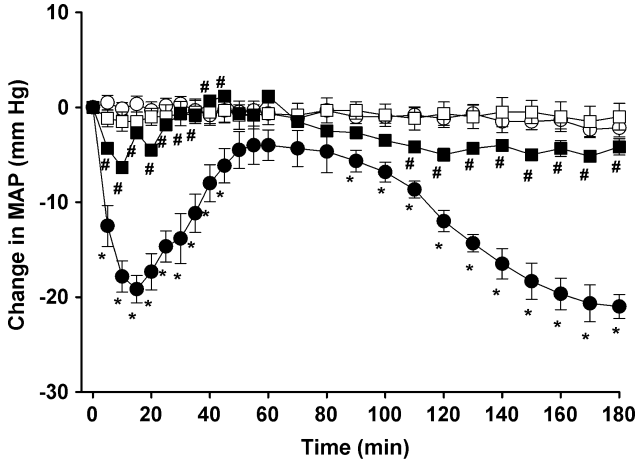

Fig. 2 Blockade of delta opioid receptors in the ventrolateral midbrain periaqueductal gray region (vlPAG) inhibits lipopolysaccharide (LPS) hypotension. Naltrindole (2 nmol) or saline (0.2 μL) was injected bilaterally into the caudal vlPAG of isoflurane-anesthetized rats and, 2 min later, LPS (1 mg/kg) or saline was administered intravenously (n = 6). $^{*}P < 0.05$, differs from saline + saline; $^{\#}P < 0.05$, differs from saline + LPS. (–○–)Saline + saline; (–●–) saline + LPS; (–□–) naltrindole + saline; (–■–) naltrinodole + LPS.

Table 1 Lidocaine or naltrindole injection into the dorsolateral periaqueductal gray (dlPAG) fails to inhibit lipopolysaccharide (LPS) hypotension

	Maximum change in MAP (mmHg)	Maximum change in HR (b.p.m.)
Saline + LPS	−31.3 ± 6.7	−27.8 ± 9.1
Lidocaine + LPS	−27.9 ± 4.3	−18.9 ± 7.6
Naltrindole + LPS	−28.7 ± 5.1	−33.9 ± 9.9

Lidocaine (2%), naltrindole (2 nmol) or saline (0.2 μL) was injected bilaterally into the caudal dlPAG of isoflurane-anaesthetized rats and LPS (1 mg/kg) was injected intravenously 2 min later (n = 6). Data represent the maximum change in mean arterial pressure (MAP) or heart rate (HR).

previously.[2] Arterial pressure fell abruptly following LPS administration, reaching its nadir within 10 min, then returned toward baseline values within 40 min. A second, more prolonged decline in arterial pressure began after 60 min and arterial pressure remained below baseline for the remainder of the experiment (Fig. 1a).

To test whether the vlPAG participates in LPS-evoked hypotension lidocaine or saline was injected bilaterally into the caudal vlPAG of isoflurane-anaesthetized rats and LPS was administered i.v. 2 min later. Lidocaine inhibited the response significantly, attenuating both the initial fall in arterial pressure and the second, decompensatory phase of LPS hypotension (Fig. 1a). Lipopolysaccharide-evoked hypotension was inhibited significantly by both 0.2 μL (Fig. 1a) and 1.0 μL of 2% lidocaine whereas 0.1 μL lidocaine was not significantly inhibitory (data not shown). Lidocaine had no effect on arterial pressure in control animals (Fig. 1a) and neither lidocaine nor LPS altered heart rate significantly (Fig. 1b).

Next, we tested whether delta opioid receptors participate in the response by microinjecting naltrindole (2 nmol) or saline into the vlPAG bilaterally. Naltrindole inhibited LPS hypotension significantly, attenuating both the first and second phases of the response, but did not change arterial pressure in control animals (Fig. 2). Neither naltrindole nor LPS affected heart rate significantly (data not shown).

In control experiments, microinjection of either lidocaine or naltrindole into the dlPAG failed to inhibit the fall in arterial pressure produced by LPS (Table 1) consistent with previous reports.[14,16] The hypotension produced by LPS can thus be prevented by inhibiting neuronal activity or by blocking delta receptors in the vlPAG, but not the dlPAG.

DISCUSSION

This study shows that microinjection of lidocaine or naltrindole into the vlPAG attenuates the fall in arterial pressure evoked by LPS. These experiments were conducted under isoflurane

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anaesthesia although earlier studies showed that anaesthesia does not alter baseline blood pressure or heart rate significantly nor does it affect the magnitude or duration of endotoxic hypotension.[2,16] These findings are predicated on earlier reports that inactivation of the POA inhibits endotoxic hypotension[2,3] and on anatomical tract-tracing studies showing that the POA densely innervates the vlPAG.[5] Functional studies also provide evidence that activation of POA neurons lowers arterial pressure through a descending pathway from the POA to the vlPAG and midline raphe nuclei.[7,8,11] The current findings thus suggest that the same, or a similar pathway, may be responsible for initiating LPS-evoked hypotension.

The concept that endotoxic hypotension is initiated through a central mechanism is by no means new. In the late 1970s Holaday and Faden[17] reported that i.v. naloxone administration inhibited endotoxic hypotension and subsequently demonstrated that naloxone was equally effective following intracerebroventricular (i.c.v.) injection.[18] Delta opioid receptors were later implicated in the response.[19] Accordingly, we found that naltrindole injection into the vlPAG inhibits the fall in arterial pressure caused by severe haemorrhage or visceral nociception.[14,16]

These findings are consistent with evidence that the vlPAG meditates 'passive' strategies for coping with trauma, predator attack, severe blood loss and deep visceral pain.[10] Activation of the vlPAG produces sympathoinhibition, hypotension, behavioural quiescence and opioid analgesia whereas activation of the dlPAG triggers 'active' coping strategies and causes sympathoexcitation, hypertension, hyperlocomotion and non-opioid analgesia.[7,10] The present data suggest that the vlPAG also participates in the cardiovascular response to endotoxemia although the rest of the pathway that mediates this response remains to be elucidated.

The central mechanism responsible for haemorrhagic hypotension has been investigated more thoroughly, however. During progressive haemorrhage, arterial pressure is initially maintained by a compensatory increase in sympathetic activity but, if blood loss becomes severe, a second, decompensatory phase ensues in which sympathetic nerve activity is inhibited and arterial pressure falls.[20,21] The decompensatory phase of haemorrhage is initiated through a descending pathway from the arcuate nucleus[22] that inhibits sympathetic nerve activity through sequential connections to the vlPAG, midline raphe nuclei and ventrolateral medulla.[10–12] A common descending pathway thus appears to mediate the cardiovascular effects of both haemorrhage and endotoxin.

The hypothesis that endotoxic hypotension is initiated by the same pathway as hemorrhagic hypotension implies that, like haemorrhage, LPS may initially lower arterial pressure by inhibiting sympathetic activity. Indeed, previous work has shown that LPS lowers arterial pressure and produces parallel reductions in splanchnic and renal nerve activities at the same dose (1 mg/kg i.v.) used in our experiments.[23,24] Lipopolysaccharide increases sympathetic activity at sub-hypotensive doses, however, which presumably maintains arterial pressure by counteracting the peripheral vasodilatory effects of LPS.[25] Interestingly, Martelli *et al.*[26] showed that transecting the splanchnic sympathetic nerves of rats treated with a sub-hypotensive dose of LPS (60 μg/kg i.v.) abruptly elevated plasma tumour necrosis factor (TNF)-α concentrations and lowered arterial pressure. This implies that sub-hypotensive doses of LPS increase sympathetic nerve activity, which suppresses the release of TNF-α and prevents the fall in arterial pressure that would otherwise occur. These findings support the hypothesis that LPS produces a biphasic response; low doses increase sympathetic nerve activity to maintain arterial pressure whereas high doses inhibit sympathetic outflow, allowing arterial pressure to fall.

Nonetheless, other data contradict this simple model. Vaysettes-Courchay *et al.*[27] showed that i.v. infusion of either 1 or 20 mg/kg LPS increased renal sympathetic nerve activity but lowered arterial pressure in anaesthetized rats, for example, and Pålsson *et al.*[28] reported that bolus injection of 20 mg/kg endotoxin produced a similar response in conscious animals. This literature is complicated by differences in species, anaesthesia and LPS doses and there is an obvious need for a comprehensive study of the relationship between LPS dose, arterial pressure and sympathetic nerve activity.

In conclusion, although the central mechanism that initiates endotoxic hypotension remains to be fully elaborated, the present data indicate that delta opioid receptors in the vlPAG are importantly involved in the response. These data contribute to a growing body of evidence that the vlPAG is a critical component of a descending pathway that integrates the behavioural and autonomic responses to severe blood loss, visceral pain and endotoxemia.

METHODS

Surgical procedures

Male Sprague-Dawley rats (250–300 g; Charles River Laboratories, Wilmington, MA, USA) were anaesthetized with 4% isoflurane and maintained with 1.5% isoflurane in 100% O_2. The left femoral artery and left jugular vein were cannulated with PE-50 tubing filled with heparinized saline (100 U/mL), the arterial cannula was connected to a volumetric pressure transducer attached to a DA100C transducer amplifier (Biopac Systems, Goleta, CA, USA) and arterial pressure and heart rate were monitored using a Biopac MP150 system. Animal protocols were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Stereotaxic injections

For intra-vlPAG injections, isoflurane-anaesthetized rats were mounted in a stereotaxic frame and lidocaine (2%; 0.1, 0.2 or 1.0 μL), naltrindole (2.0 nmol; 0.2 μL) (Sigma Chemical Co., St. Louis, MO, USA) or an equivalent volume of saline was injected bilaterally into the vlPAG with a Hamilton syringe connected to cannulae lowered vertically 0.8 mm lateral and 8.3 mm caudal from bregma and 6.2 mm below the dural surface.[29] For dorsolateral PAG (dlPAG) injections, 2% lidocaine, 0.2 nmol naltrindole or 0.2 μL saline was injected bilaterally 0.8 mm lateral and 8.3 mm caudal to bregma and 4.6 mm below the dural surface.[14,16]

Lidocaine and naltrindole were dissolved in saline containing 0.2% Chicago Sky Blue dye to mark the injection sites. Drug doses were selected from dose-response studies published previously.[13,16] Stereotaxic injections were delivered at a constant rate over a 1-min period and, 2 min later, LPS (1.0 mg/kg) or saline (1 mL/kg) was administered i.v. At the end of each experiment,

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rats were killed with an overdose of isoflurane, brains were removed, sectioned (50 μm) and stained with eosin to confirm the location of cannulae. Only data from confirmed cannula placements were included in this report.

Statistical analysis

Data are reported as mean ± standard error of the mean (SEM) and were analyzed by repeated measure two-way ANOVA followed by Bonferroni's multiple comparison test. A two sided P value of < 0.05 was considered significant.

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