Comparison of the Effects of Intra-Third Ventricular Administration of Interleukin-1 or Platelet Activating Factor on ACTH Secretion and the Sympathetic-Adrenomedullary System in Conscious Rats

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The effects of centrally administered interleukin-1β (IL-1) or platelet activating factor (PAF) on adrenocorticotropic (ACTH) and catecholamine secretion, blood pressure and heart rate were examined to determine if these agents stimulate similarly the hypothalamic-pituitary-adrenal (HPA) axis or the sympathetic-adrenomedullary system. Intra-third ventricular administration of IL-1 (50, 200 ng) evoked significant ACTH secretion. Centrally administered IL-1 (50 ng) elevated plasma noradrenaline and adrenaline levels, systolic blood pressure and heart rate. Plasma ACTH, noradrenaline and adrenaline levels were also increased by the higher dose (200 ng) of IL-1 while systolic blood pressure and heart rate were not affected. Intra-third ventricular administration of 9 μg of PAF elevated the plasma ACTH level while 3 μg of PAF did not stimulate ACTH secretion. Neither dose of centrally administered PAF affected any plasma catecholamine level or systolic blood pressure. These results suggest that central IL-1 stimulates both the HPA axis and the sympathetic-adrenomedullary system, that a higher dose of IL-1 stimulates a mechanism to antagonize the elevation of blood pressure and heart rate and that central PAF is not involved in the control of the sympathetic-adrenomedullary system. Thus, IL-1 and PAF do not interact in the brain, although they interact peripherally.

Key words: interleukin-1, platelet activating factor, ACTH, noradrenaline, adrenaline

Evidence is accumulating for the existence of communication between the immune and neuroendocrine systems. The stress-induced products of the hypothalamic-pituitary adrenal (HPA) axis, namely, adrenocorticotropic (ACTH) and cortisol, have been shown to influence immune responses (1). Mediators of inflammation can activate both central and peripheral components of the HPA axis. Interleukin-1 (IL-1), an immune cytokine, has been shown to affect the HPA axis (2–9) and platelet-activating factor (PAF) has been shown to modulate the function of both the immune and endocrine systems (10–12). PAF is a naturally occurring phospholipid which serves as a potent mediator of inflammation and analgesia (13) and causes platelets to aggregate. Also, PAF is produced in the brain (14, 15) and the brain contains receptor for PAF (16). And PAF is known to influence neural function and development as well as neuroendocrine function through platelet-independent processes (11).

In peripheral cells, IL-1 and PAF are interrelated. PAF stimulates IL-1 release by human monocytes (17) and IL-1 stimulates PAF production by human monocytes (18) and cultured human endothelial cells (19). These reports led us to speculate that IL-1 and PAF may also interact in the brain. Both peripherally and centrally administered IL-1 and PAF have been reported to stimulate the HPA axis. These mediators have been demonstrated to stimulate corticotropin-releasing hormone

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(CRH) synthesis and secretion (6, 20-23). Centrally administered CRH has been shown to stimulate peripheral catecholamine secretion, thereby elevating blood pressure and heart rate (24). In this study, we investigated the effects of centrally administered IL-1 and PAF on peripheral catecholamine secretion, blood pressure and heart rate to determine whether IL-1 and PAF stimulate the HPA axis or the sympathetic-adrenomedullary system.

**Materials and Methods**

*Cannula implantation.* Male Wistar rats (290-350g) were anesthetized with intraperitoneal sodium pentobarbital (45mg/kg BW, Somnopenty, Pitman Moore Inc., NJ, USA) and a stainless steel guide cannula (C313G) was implanted stereotaxically in the third ventricle to allow direct injection of IL-1 and PAF into the cerebrospinal fluid. The rats were caged individually and received standard rat biscuits and water ad libitum. Five or six days later, the femoral artery was cannulated with a PE50 polyethylene tube (Intramedic, Clay Adams, USA) under pentobarbital anesthesia. The catheter was tunneled under the skin to exit at the nape of the neck. Two days later, the experiments were carried out in a quiet room.

*Experiment 1.* About 1h prior to the experiment, a PE50 polyethylene tube was connected to the cannula to collect blood samples and to monitor arterial pressure. The connecting tube and cannula were filled with 0.9% saline containing heparin sodium (500U/ml) and the end of the tube was suspended outside of the cage. Thus, the rats were able to move freely. Thirty min later, 1 ml of blood was withdrawn from the cannula into a heparinized syringe and was replaced with 1 ml of saline. Plasma was separated for catecholamine measurement and blood cells were resuspended in saline and mixed for later replacement. Then, the cannula was connected through a pressure monitoring kit (SCK-590, Spectramed Medical Products, Ltd, Tokyo, Japan) to a Nihon Koden recorder (connection board, RPM-6004; blood pressure amplifier, WT-625G; Tokyo, Japan) for continuous measurement of arterial pressure and heart rate. Thirty min later, 0.3 ml of blood was collected for baseline ACTH assay and was replaced by 0.3 ml of saline. Three microliters of vehicle or IL-1 (50 and 200ng/3μl; Otsuka Pharmaceutical Co., Tokushima, Japan) was injected into the third ventricle. Ten, 30 and 60 min later, 1.3 ml of blood was collected. Plasma was separated for catecholamine and ACTH measurement and blood cells were resuspended in saline and mixed. Then, resuspended blood cells (1.3 ml) were replaced at 10, 30 and 50 min. Blood pressure monitoring was continued until 30 min after injection.

*Experiment 2.* To investigate the central action of PAF, experiment 2 was carried out as was in experiment 1 except that vehicle (3μl) or PAF (3 and 9μg/3μl, 1-o-hexadecyl-2-acetyl-sn-glycero-3-phosphoryl-choline, Bachem, USA) was injected into

The third ventricle and blood samples were collected at 10, 30, 60 and 120 min after injection. Plasma catecholamine levels were measured also until 120 min after injection, as the levels did not change during the first 30 min in a preliminary experiment.

**Hormone assay.** Blood samples were collected in a chilled plastic tube and centrifuged (1,200 × G) at 4°C and plasma was stored at −20°C pending assay. The plasma ACTH concentration in 50μl of plasma was measured with a commercially available radioimmunoassay kit (Mitsubishi Yuka Co., Tokyo, Japan). Plasma noradrenaline and adrenaline concentrations were determined by ion-pairing reverse-phase high performance liquid chromatography with amperometric detection. The details of this catecholamine assay have been reported (24).

**Statistical analysis.** Values are presented as the mean ± SEM. The data were evaluated statistically by analysis of variance followed by Duncan’s new multiple range test.

**Results**

**Experiment 1.** Intra-third ventricular administration of IL-1 (50, 200 ng) evoked significant ACTH secretion at 10 min after injection (Fig. 1). Plasma ACTH remained elevated for 60 min. Centrally administered IL-1 (50 ng) elevated plasma noradrenaline levels from 10 to 60 min after injection (Fig. 2). The higher dose (200 ng) of IL-1 also elevated plasma noradrenaline levels, however, this was significant only at 10 min. Plasma adrenaline was elevated by 50 ng of IL-1 at 10 and 30 min after injection.

![Fig. 1](https://via.placeholder.com/150)

**Fig. 1** Effect of intra-third ventricular administration of interleukin-1 (IL-1) on plasma ACTH levels in conscious rats. Bars represent the mean ± SEM vehicle (n = 7); IL-1, 50 ng (n = 6); and IL-1, 200 ng (n = 7). *, P < 0.01 vs. vehicle; **, P < 0.01 vs. baseline levels.
Fig. 2  Effect of intra-third ventricular administration of IL-1 on plasma noradrenaline and adrenaline levels in conscious rats. Bars represent the mean ± SEM. Vehicle (n = 5); IL-1, 50 ng (n = 6); and IL-1, 200 ng (n = 7). +, P < 0.05, ++, P < 0.01 vs. vehicle; *, P < 0.05; and **, P < 0.01 vs. baseline levels.

Fig. 3  Effect of intra-third ventricular administration of IL-1 on systolic blood pressure and heart rate in conscious rats. Bars represent the mean ± SEM. Vehicle (n = 6 for blood pressure and n = 7 for heart rate); IL-1, 50 ng (n = 7); IL-1, 200 ng (n = 10). +, P < 0.05, ++, P < 0.01 vs. vehicle; **, P < 0.01 vs. baseline levels.

while 200 ng of IL-1 elevated adrenaline levels at 30 and 60 min after injection. Centrally administered IL-1 (50 ng) increased arterial blood pressure from 10 to 30 min after injection whereas 200 ng of IL-1 did not elevate blood pressure (Fig. 3). Heart rate was increased by 50 ng of IL-1 from 10 to 20 min after injection whereas 200 ng of
IL-1 did not affect heart rate.

Experiment 2. Intra-third ventricular administration of 9 μg of PAF elevated plasma ACTH levels at 10 min (Fig. 4). Plasma ACTH remained elevated for 60 min. However, 3 μg of PAF did not stimulate ACTH secretion. Centrally administered PAF (3 or 9 μg) affected neither plasma catecholamine levels (Fig. 5) nor systolic blood pressure (data not shown).

Discussion

Intra-ventricular IL-1 injection dose-dependently evoked ACTH secretion, thereby confirming previous reports. It has been reported that peripheral administration of IL-1 increases the level of CRH mRNA in the hypothalamus (25) and that IL-1 stimulates directly in vitro the release of CRH from rat hypothalamus (6, 23). Rivier et al. (26) reported that centrally administered IL-1 stimulated catecholamine secretion. Our results also show that centrally administered IL-1 increases plasma noradrenaline and adrenaline levels. However, a dose-response relationship was not found in the range between 50 and 200 ng of IL-1. The reason for this dose-independency is difficult to explain, however, it might be ascribed to the levels of plasma catecholamine stimulated by 50 and 200 ng of IL-1 were near the maximum levels which could be stimulated by central IL-1. Centrally administered IL-1 (50 ng) elevated both systolic blood pressure and heart rate, whereas 200 ng of IL-1 affected neither systolic blood pressure nor heart rate. These results suggest that a relatively small dose of centrally administered IL-1 stimulates the peripheral sympathetic-adrenomedullary system to secrete noradrenaline and adrenaline, thereby elevating blood pressure and heart rate. Although 200 ng of IL-1 stimulated the peripheral sympathetic-adrenomedullary system, no significant cardiovascular change was observed. These results suggest that although central IL-1 stimulates ACTH secretion and the peripheral sympathetic-adrenomedullary system, probably via CRH stimulation, IL-1 at a higher dose may also stimulates a mechanism to antagonize the blood pressure and heart rate elevation. Parasympathetic stimulation may be involved in this mechanism.

It has been reported that IL-1 stimulates PAF production by human monocytes (19). We speculated that IL-1 may also stimulate PAF production in the brain and that central PAF may be involved in peripheral or central IL-1-induced CRH activation. Although, in the present study, a relatively large dose of centrally administered PAF stimulated ACTH secretion, it did not stimulate the peripheral sympathetic-adrenomedullary system. A rela-
tively low dose of PAF stimulated neither ACTH secretion nor catecholamine secretion and centrally administered PAF did not affect systolic blood pressure. These results suggest that central PAF is not involved in controlling cardiovascular function although peripheral PAF evokes hypotension by decreasing myocardial contractility and heart rate and by causing vasodilation (27). Therefore, it is unlikely that central PAF is involved in peripheral and central IL-1-induced activation of CRH secretion. IL-1 and PAF may activate ACTH secretion by independent mechanisms. Thus, IL-1 and PAF may not interact in the brain, although they interact peripherally. The physiological significance of central PAF-stimulated ACTH secretion remains uncertain as PAF thought to be neurotoxic at high concentration (11).

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