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Effects of Physical Stress and Cytokines on Hypothalamus, Pituitary and Adrenal Glands
(ストレスおよびサイトカインの視床下部、下垂体、副腎皮質ホルモン分泌およぼす影響について)

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主論文①

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INTERLEUKIN-1β ANALOGUES WITH MARKEDLY REDUCED PYROGENIC ACTIVITY CAN STIMULATE SECRETION OF ADRENOCORTICOTROPIC HORMONE IN RATS

Yoshiyuki Naito[1], Junichi Fukata[2]*, Yoshihiro Masui[3], Yoshikatsu Hirai[3], Norihiko Murakami[2], Tomoko Tominaga[2], Yoshikatsu Nakai[2], Sunao Tamai[1], Kenjiro Mori[1] and Hiroo Imura[2]

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主論文②

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ADRENOCORTICOTROPIC HORMONE-RELEASING ACTIVITIES OF INTERLEUKINS IN A HOMOLOGOUS IN VIVO SYSTEM

Yoshiyuki Naito[1], Junichi Fukata[2], Tomoko Tominaga[2], Yoshihiro Masui[3], Yoshikatsu Hirai[3], Norihiko Murakami[2], Sunao Tamai[1], Kenjiro Mori[1] and Hiroo Imura[2]

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主論文③

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INTERLEUKIN-6 STIMULATES THE SECRETION OF ADRENOCORTICOTROPIC HORMONE IN CONSCIOUS, FREELY-MOVING RATS

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Chronic Effects of Interleukin-1 on Hypothalamus, Pituitary and Adrenal Glands in Rat

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EFFECTS OF INTERLEUKINS ON PLASMA ARGININE VASOPRESSIN AND OXYTOCIN LEVELS IN CONSCIOUS, FREELY-MOVING RATS

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SUMMARY: To elucidate whether interleukins are involved in vasopressin or oxytocin release during cytokine-related stressful conditions, we examined the effects of human interleukin-1 β and interleukin-6 on plasma vasopressin and oxytocin levels in rats. Interleukin-1 β administrated intravenously stimulated both the vasopressin and oxytocin secretion in dose-dependent manners. Neither hormone release was observed following interleukin-6 administration. Pretreatment with aspirin significantly attenuated the effets of interleukin-1 β on both the vasopressin and oxytocin levels. SC-19220, a prostaglandin E_2 receptor antagonist, did not affect the interleukin-1 β -induced increase of plasma oxytocin levels, but almost completely abolished its effect on plasma vasopressin levels. These results suggest that under certain stressful conditions which accompany the stimulation of cytokine production, interleukin-1 is involved in the increace of plasma vasopressin and oxytocin levels and, moreover, different kinds of prostaglandins are suggested to participate in these interleukin-1-induced hormone release.

AVP and OXY are reported to increase in plasma after the live bacterial challenge or iv administration of endotoxin (1,2). Under such conditions, it has been known also that several cytokines increase either in the general circulation or locally. Among the cytokines, IL-1 and IL-6 are suggested to be major ones in causing endocrine and metabolic abnormalities associated

with these conditions (3,4). To ascertain the possible roles of IL-1 and IL-6 in such invasive stimuli-induced elevation of plasma AVP and OXY levels, we administered rhIL-1β and rhIL-6 iv to conscious, freely-moving rats and observed the changes of plasma AVP and OXY levels measured by RIA. In addition, we studied whether PGs mediate cytokine-induced changes of plasma AVP and OXY levels.

MATERIALS AND METHODS

Materials and experimental protocol

Adult male rats of the Wistar strain, weighing 300-350g, were kept under a controlled temperature (25 ± 1 ℃) and fixed light-dark schedule (light on at 0600 h, off at 1800 h). Food and water were provided ad libitum. Several days before blood sampling, a silastic cannula was set in the right atrium through the jugular vein and a stainless steel guide cannula (20 G) was implanted stereotaxically and secured with dental cement at 0.8 mm posterior, 1.4 mm lateral, 3.0 mm ventral to the Bregma to facilitate injection into the right lateral ventricle.

At least 12 hours prior to blood sampling, which routinely began at 0945 h, cannulated animals were moved to a special sampling cage in which blood could be drawn from the rats under conscious, freely-moving conditions without apparent stress throughout the experimental period (4). Two hours before blood sampling, an icv injection cannula (24 gauge) was inserted through the guide cannula to place the tip in the anterior horn of the right lateral ventricle.

Biological activities of rhIL-1β and rhIL-6 used in this experiment were reported previously (3,5). Aspirin was administered as a water soluble form (Venopirin; The Green Cross Corporation, Osaka, Japan). The effects of PG receptor antagonists on cytokine-induced release of AVP and OXY were assessed by continuous icv administration of the following materials: BW A868C, a PGD_2 receptor (DP receptor) antagonist (6), and SC-19220, a PGE_2 receptor (EP_1 receptor) antagonist(7), both of which were generously provided by The Wellcome Foundation Ltd. Beckenham, UK, and Searle Research and Development, Skokie, IL, respectively.

Cytokines were dissolved in 0.2 ml of 0.9% saline containing 0.1% bovine serum albumin and were iv injected through the intra-atrial cannula. Control rats for each experiment received iv and/or icv injection with only the vehicle. Blood samples (0.6 ml) were withdrawn into heparinized syringes from the same cannula before (-15 and 0 min) and 15, 30, 60, 90, and 120 min after the injection of cytokine. Blood samples were immediately cooled on ice and then centrifuged to obtain the plasma samples (0.25 ml) which were stored at -20 ℃ until hormone assay. Plasma osmolarity was also determined

by measuring the freezing point using the specimen obtained similarly. Red cells were resuspended in physiologic saline and returned to the rats after each sampling.

Hormone assay

After extraction using Sep-Pak C_{18}, plasma AVP levels were determined by RIA using AVP RIA kits purchased from Mitsubishi Petrochemical Co., Ltd., Tokyo, Japan (8). The recovery ratio of synthetic AVP added to rat plasma by this extraction procedure was 90% and the results were not corrected by this value. The minimal detectable quantity of plasma AVP by this assay system was 0.6 pg/ml and intra- and interassay coefficients of variation were both less than 10%.

Plasma OXY levels were measured by RIA using anti-OXY rabbit antiserum without extraction, as described previously (2). The minimal detectable quantity of plasma OXY by this system was 5 pg/ml and intra- and interassay coefficients of variation were both less than 10%.

Statistical analysis

Statistical analysis was performed by the ANOVA or the repeated measurements ANOVA and subsequent Bonferroni method; $p<0.05$ was considered to be significant. Results were expressed as the mean $\pm$ SEM of 5 rats.

RESULTS

Prior to the measurement of the hormone levels, a couple of preliminary experiments were performed to evaluate the validity of experimental system. First, we measured the levels of plasma osmolarity and found that osmolarity showed only minimal changes throughout the sampling period even in the rats which received the highest dose of rhIL-1β, that is, the mean ($\pm$ SEM) basal level at 0 min and the maximal level at 60 min were 292 $\pm$ 0.8 and 294 $\pm$ 1.0 mOsmol/kg, respectively. Second, we observed the changes of blood pressure during the IL-1β challenge and ascertained that iv administration of rhIL-1β did not significantly change the systemic arterial pressure even at the highest dose of 10 μg (Figure 1).

Effects of rhIL-1β and rhIL-6 administrated iv on plasma AVP and OXY

levels were assessed after these preliminary experiments. As shown in Figure 2, rhIL-1 β significantly elevated both plasma levels of AVP and OXY in dose-dependent manners. The minimal amount of rhIL-1 β which could elicit AVP and OXY increase were 1 μ g and maximal plasma AVP and OXY levels were usually observed 30 min after the rhIL-1 β administration. On the other hand, neither AVP nor OXY levels in plasma were affected by the iv administration of rhIL-6 even when 10 μ g was administered. Both the hormone levels of vehicle-injected control rats showed no significant changes during the sampling period.

We next examined the possible involvement of PGs in IL-1 β -induced AVP and OXY secretion. Effects of a cycloxygenase inhibitor, aspirin, and PG receptor antagonists, BW A868C and SC-19220, on rhIL-1 β -induced elevation of plasma AVP and OXY levels were summerized in Figure 3. Aspirin which did not alter the basal plasma levels significantly attenuated the rhIL-1 β -induced elevation of both plasma AVP and OXY levels. While icv treatment of SC-19220, a EP_2 receptor antagonist, completely abolished the rhIL-1 β -induced elevation of plasma AVP level, plasma OXY response to rhIL-1 β did not affected by this treatment. Similar administration of a DP receptor antagonist, BW A868C, had no effect on the increase of plasma AVP and OXY levels induced by rhIL-1 β.

DISCUSSION

Accumulating evidence suggests the existence of bidirectional communication between the immune and neuroendocrine system. Among the neuroendocrine changes, the release of posterior pituitary hormones during

immune or inflammatory processes has not yet been well examined. Recently, IL-1 and IL-6, which play similar roles in many metabolic responses to acute phase inflammatory reactions (9,10), were reported to show similar stimulatory effects on the hypothalamo-pituitary functions both in vivo and in vitro (11). The present study demonstrated that IL-1 administered iv increased plasma levels of AVP and OXY without apparent changes of plasma osmolarity and arterial blood pressure. On the other hand, IL-6 has no such effects under the same conditions. In 1987, Sapolsky et al reported that both AVP and OXY levels in the pituitary portal blood did not significantly change after iv administration of IL-1, the amount of which was sufficient to cause ACTH release (12). Therefore, the present data are the first observation showing that a cytokine does increase the plasma levels of posterior pituitary hormones. Our data concerning IL-1 are consistent with a previous report using an in vitro perifusion experiment (13) and suggest that, during inflammation, not IL-6 but IL-1 mediates the effects of inflammatory reactions on posterior pituitary resulting in the hormone release without either significant hemodynamic or osmolarity change. This idea is compatible with the hypothesis that IL-1 and IL-6 do not always share similar actions on the neuroendocrine organs during the acute phase inflammatory reaction.

The present study also suggest that the stimulatory effects of IL-1 on AVP and OXY secretion are dependent on PG synthesis. PGE_2 and PGD_2 were reported to increase plasma AVP and OXY levels (14) and possible involvement of PG as a mediator of IL-1-induced responses of the central nervous system has already been postulated (15,16). Our present reports indicated also that PGE_2 plays an important role in the AVP release induced by IL-1. In terms of

IL-1-induced OXY release, however, neither DP- nor EP_1-receptor antagonist showed significant effects. This suggests that PGE_2 and PGD_2 are not involved in IL-1-induced OXY release. $PGF_{2\alpha}$, another PG found in the brain, might be responsible for the IL-1-stimulated OXY secretion as was suggested from the previous report (17). Further study should clarify the mediator in IL-1-induced OXY release.

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FOOTNOTES

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Abbreviations: ACTH, adrenocorticotropic hormone; AVP, arginine vasopressin; icv, intracerebroventricular(ly); iv, intravenous(ly); rhIL-1β, recombinant human interleukin-1 β; RIA, radioimmunoassay; OXY, oxytocin; PG, prostaglandin.

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FIGURE LEGENDS

Figure 1. Effects of iv administration of rhIL-1β (10 μg) on systemic arterial blood pressure (SBP, shaded area) and heart rate (HR, ●). Rats were catheterized in the left femoral vein (for administration of cytokines) and femoral artery (for recording blood pressure and heart rate) under the halothane anesthesia (2%) with artificial ventilation through a tracheal cannula. Each point represents the mean of 3 rats.

Figure 2. Time-course of the changes of plasma AVP (upper panel) and OXY (lower panel) levels in response to iv administration of rhIL-1β or rhIL-6. The cytokines were administered at the following doses: (□):rhIL-1β 10 μg, (○):rhIL-1β 1 μg, (△):rhIL-1β 0.1 μg, (▲):rhIL-6 10 μg, (●):control. Each point represents the mean ± SEM of 5 rats. *:$p<0.05$ compared to the control rats.

Figure 3. Effects of aspirin, BW A868C or SC-19220 on the changes of plasma AVP (upper panel) and OXY (lower panel) levels induced by rhIL-1β. Aspirin was administered 30 min and immediately before the rhIL-1β administration at a dose of 100 mg/kg for each injection. Continuous icv administration of BW A868C or SC-19220 was started 120 min before the cytokine injection and continued throughout the sampling period at the doses of 10 μg/kg/min and 50 ng/kg/min, respectively, which are reported to be effective in in vivo experiments (6,7). Maximal values of these hormone levels were usually observed 30 min after the cytokine injection. Each column represents the mean ± SEM of the 5 rats. *:$p<0.05$ compared to the control rats.

Fig 1

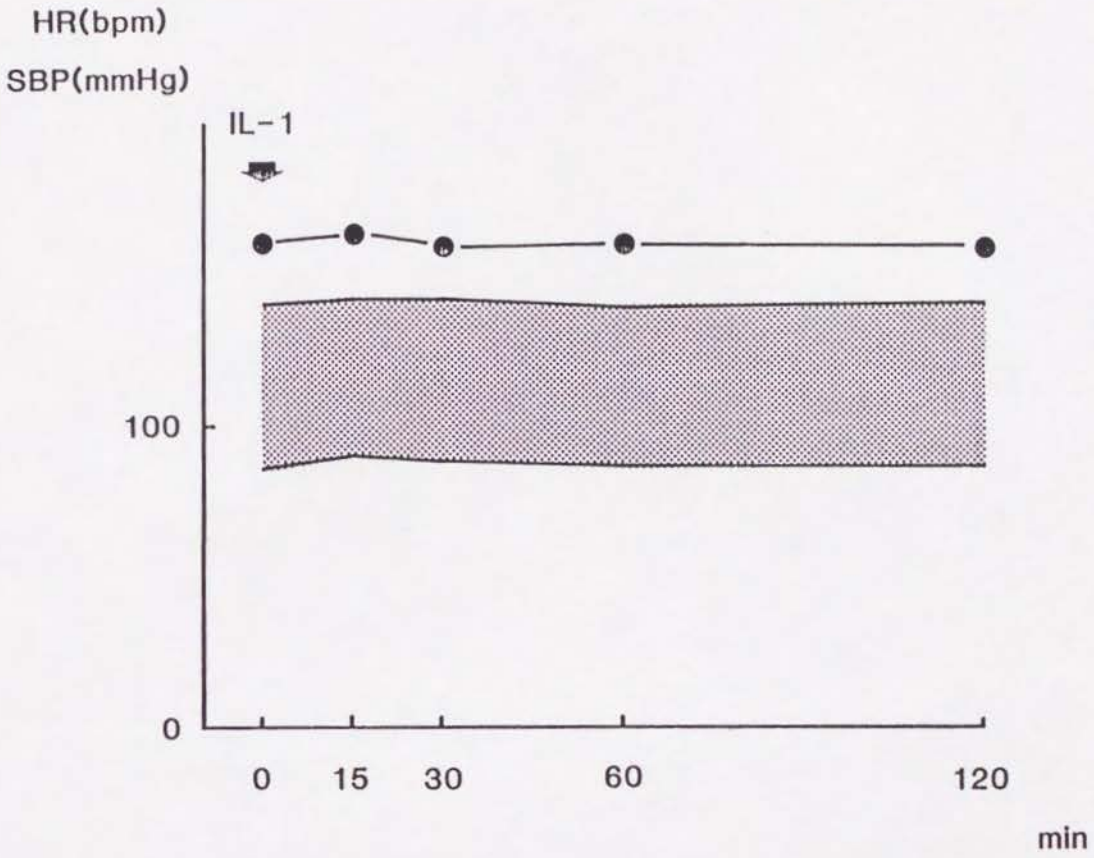


Fig 2

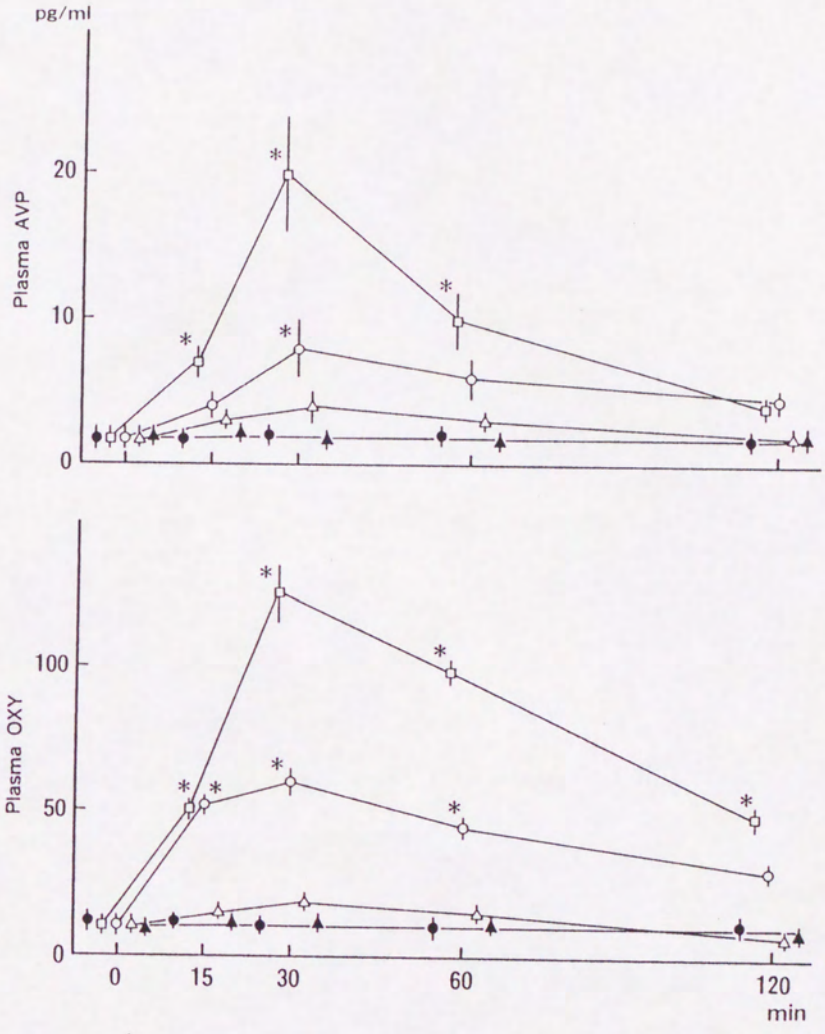
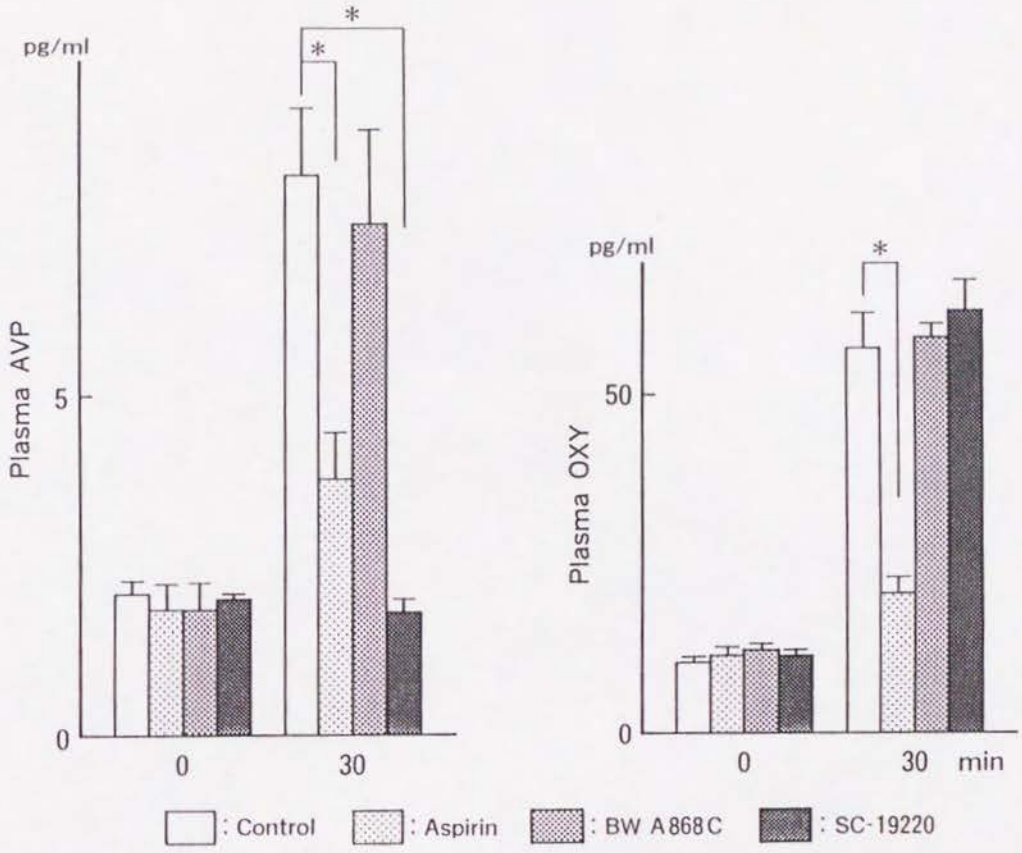


Fig 3


pg/ml
Plasma AVP
5
0
0
30
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pg/ml
Plasma OXY
50
0
0
30
min
*
: Control
: Aspirin
: BW A868C
: SC-19220