Endotoxin Injection Increases Growth Hormone and Somatostatin Secretion in Sheep

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ABSTRACT

Endotoxin has been shown to stimulate GH secretion in human and sheep. However, changes in hypothalamic neurohormones involved in the GH regulation by endotoxin have never been studied in vivo. In sheep it is possible to collect hypophysial portal blood (HPB) and quantify GH-releasing hormone (GHRH) and somatostatin (SRIH) secretion under physiological conditions. The purpose of this study was to determine the effect of an acute iv endotoxin administration on the secretion of these peptides in sheep.

Endotoxin induced a sustained increase of GH (×6.2 ± 1.3) in intact rams. This stimulation was delayed and less marked when compared with the hypothalamic-pituitary-adrenal axis. Surprisingly, the GH increase was associated with an important rise of jugular (×10.6 ± 2.4) and portal (×7.9 ± 3) SRIH levels, without a significant GHRH increase. To determine if the portal SRIH increase was a consequence of an increased short feedback of GH, we studied GH response to endotoxin after a previous GHRH injection to deplete the pituitary pools of GH. In that case, despite the absence of increase of GH after endotoxin treatment, SRIH levels were markedly increased. For the first time we have observed an experimental situation in sheep with a simultaneous and closed amplitude increase in jugular and portal SRIH. The source of jugular SRIH is likely the gastrointestinal tract and the increased jugular SRIH release in systemic circulation might be in part responsible for the increase of hypophysial portal SRIH.

Ultimately our results show that endotoxin induced a complex reaction at multiple levels with a specific increase in both portal and peripheral SRIH levels. The surprising association of a lack of change in GHRH release and an increased secretion of SRIH with the increase of GH suggests that the effect of endotoxin on GH axis is mainly in the pituitary one. The selective blockade of somatostatin should be useful for a better knowledge of the role of SRIH stimulation in the physiopathology of septic shock. (Endocrinology 139: 2662–2669, 1998)

T HE NEUROENDOCRINE system plays an important role in maintaining homeostasis under a variety of stress conditions including microbial infection and endotoxin shock. Endotoxin, a lipopolysaccharide found as principal component of gram-negative bacteria (LPS), is the main mediator of septic shock (1). When administered in vivo LPS, strongly stimulates the immune system as well as it modulates the secretion of several mediators and hormones. Briefly, LPS induces a rapid increase in plasma concentration of tumor necrosis factor-α (TNFα), interleukin 1 (IL-1), and interleukin 6 (IL-6). In turn, endotoxin together with circulating cytokines stimulates prostaglandin E2 (PGE-2) and interleukin production by various tissues including the hypothalamus and the pituitary gland; its action is mediated, at least in part, via CD14, an endotoxin receptor, which has been identified in monocytes, serum (as a soluble form) as well as in brain and in pituitary (2, 3). Changes in the secretion of several hormones have also been observed (4). Several teams have observed that hormonal changes induced by LPS are important and occur at multiple levels. Indeed, LPS increases activity of the hypothalamic-pituitary-adrenal (HPA) axis (5–7) and decreases TSH (8) and LH (9–11) secretion. The activation of the pituitary adrenal axis after endotoxin injection is associated with an increased release of both corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) secretion into hypophysial portal blood (7). The gonadal effect of endotoxin and interleukins occurs also at multiple levels (hypothalamic, pituitary and gonadal), the dramatic decrease of LH being associated with a decrease of GnRH gene expression (12, 13). Communications between the immune and endocrine systems are bi-directional and the hormonal changes induced by endotoxin or cytokines can act on the immune system.

The somatotropic axis is also involved in the reaction to endotoxin or cytokines administration. Indeed, GH and insulin-like growth factor I (IGF-1) have been identified in immune competent cells with immunomodulatory properties (14). The administration of endotoxin induces species-dependent effects on GH secretion: indeed, it increases GH secretion in humans (15, 16) and in sheep (17) but decreases it in rats (18) and cattle (19). The mechanisms of action of endotoxin are not clearly understood. The action of endotoxin may be exerted directly at the level of the pituitary gland or indirectly at the level of one or both hypothalamic neurohormones GH-releasing hormone (GHRH) and somatostatin (SRIH), which act respectively as stimulus or in-
hibitor of GH secretion. In vitro, endotoxin increases basal GH release from dispersed sheep anterior pituitary cells in a time- and dose-dependent manner (17, 20). Moreover, TNFα in sheep (21) and IL-6 in rats (22) have been shown to act on GH secretion directly at the pituitary level. However, the direct effect of IL-1 on GH secretion from the pituitary gland is unclear with contradictory reports (23–25). The action of IL-1 at the level of the hypothalamus has been tested in rats. IL-1 stimulates GHRH and SRIH release from hypothalamic explants (26). Furthermore in rats, the increase of GH induced by the intracerebroventricular inject 10 ng of IL-1 is abolished by the immunoneutralisation of GHRH (27).

There are experimental limitations in rodent models and growing interest in a clinical relevant animal model, such as the sheep, for GH regulation (28). In this animal, it is possible to collect hypophysial portal blood (HPB) and quantify the secretion of neurohormones into HPB under physiological conditions. The purpose of this study was to determine the effects of an acute iv administration of endotoxin on jugular GH, portal GHRH, and jugular and portal SRIH plasma levels in intact rams chronically implanted with perihypophysial cannulae.

Materials and Methods

Animals

Fourteen intact rams (9–11 months old, 40–45 kg BW) from the Merinos Alps breed were obtained from Ecole Nationale Superiéure Agronomique, Domaine du Merle, Salon de Provence (France). Two weeks before the onset of the study, the rams were transferred to the animal room of the laboratory. All experimental procedures were performed in accordance with local animal use regulations; studies were approved by the Faculty Committee on the Use and Care of Animals.

Experimental procedures

Three sets of experiments were carried out successively. For each experiment, endotoxin (Escherichia coli 055: B5; Sigma, St. Louis, MO) was reconstituted in PBS with 0.1% BSA to a stock concentration of 0.1 μg/μl. Body temperature was recorded regularly.

Exp 1. The aim of this experiment was to study the effect of endotoxin on plasma GH levels in intact rams and to compare the chronology of GH changes with those of several components of the hypothalamic-pituitary-adrenal (HPA) axis. The animals (n = 6) were housed in individual pens placed immediately adjacent to each other. They were free to sit or to stand, exposed to natural lighting conditions and allowed free access to food and water. An indwelling catheter was inserted into the external jugular vein. On the following day, endotoxin was administered by an iv bolus injection (400 ng/kg) in 2 ml of saline through the jugular cannula, followed by 5 ml of saline as previously described (16). Jugular blood was collected every 15 min during the 2-h period preceding endotoxin injection and during the subsequent 9 h. Blood samples were immediately centrifuged at 4 °C for 10 min and the resulting plasma was stored at −20°C until assayed for GH, ACTH, cortisol, and AVP.

Exp 2. The aim of this experiment was to determine the effects of an acute iv endotoxin administration on jugular GH, portal GHRH, and jugular and portal SRIH plasma levels in intact rams chronically implanted with perihypophysial cannulae. Fifteen days before the experiment, four rams were anesthetized and prepared for portal blood sampling under general anesthesia, as previously described (29, 30). A twin cannula was implanted through the transnasal route in front of the long portal vessels, above the anterior pituitary gland. After 14 days, two catheters were inserted in each jugular vein, one catheter for injection of heparin and endotoxin, the other one for collection of peripheral blood. Two animals were placed side to side in two small pens. One day later, heparin (an initial dose of 25,000 IU followed by 5,000 IU every 30 min) was injected and at 0600 h, a needle was inserted into the upper cannula to create a lesion of the hypophysial portal vessels. The resulting portal blood was collected through the lower cannula.

Animals were injected with endotoxin (200 ng/kg) as described above. Portal and jugular blood was collected every 15 min during the 2-h period preceding endotoxin injection and during the subsequent 9 h. Samples were handled as described in Exp 1 until assayed for GH, SRIH, and GHRH.

Exp 3. The aim of this experiment was to determine if the increase of SRIH after endotoxin was due to an increased short feedback of GH on the hypothalamus. Four rams were prepared for portal blood sampling as described above. Two hours after the beginning of the experiment hGHRH(1–44)NH2 (1 μg/kg bw) (Sanofi, Toulouse, France) was administered iv as a bolus. Jugular and portal blood were collected every 15 min for 7 h. Samples were handled as described in Exp 1 until assayed for peripheral GH and portal SRIH.

Hormone assays

The GH RIA was performed in duplicate using reagents provided by NIADDK, Hormone Distribution Program (Bethesda, MD). oGH-1–4 was used as standard and the least detectable concentration of GH was 0.5 ng/ml plasma: the intra and interassay coefficients of variation (CV) were 7 and 11%, respectively.

Before GHRH and SRIH RIA, peptides were extracted from plasma with 2 vol acetone/20 m HCl. The SRIH RIA was performed in duplicate in portal and jugular plasma extracts using [125I]-Tyr-somatostatin as radioligand. The antiserum (no. 2044) was a gift from Dr. C. Rougeot (INSERM U207, Paris, France). The intra and interassay CV were 7 and 10%, respectively, and the least detectable concentration was 5 pg/ml plasma. The GHRH RIA was performed in duplicate in portal plasma extracts. oGHRH (Peninsula, St. Helens, Messeyxide, UK), labeled with 1125 using the lactoperoxidase method, served as radioligand. Antiserum was raised in our laboratory by immunization of rabbits against oGHRH coupled to BSA with glutaraldehyde. Intra and interassay CV were 9 and 11%, respectively, and the least detectable concentration of oGHRH was 10 pg/ml plasma. Both assays have been previously described (31).

Cortisol, ACTH, and AVP levels were measured in extracted plasma according to RIA methods previously described (32). The intraassay coefficients of variations within the measurement range of each assay were: cortisol 4.7%, ACTH 5%, AVP 5.5%. The limits of detection of the assays were 0.5 ng/ml plasma for cortisol, 10 pg/ml plasma for ACTH, 5 pg/ml plasma for AVP.

Statistical analysis

All data are reported as the mean ± SEM. In Exp 2, mean plasma GH, GHRH, and SRIH values were calculated during each 60-min period. In Exp 3, the mean plasma hormone concentrations were calculated for the period of basal secretion (2 h) the period following GHRH iv injection (2 h) the period of return to GH basal secretion (1 h), and the period following endotoxin administration (2 h). All statistical analysis were performed using one-way ANOVA for repeated measures followed by Fisher’s test (with computer program: Statview 512, Brain Power, Inc., Calabasas, CA). P < 0.05 was considered significant.

Results

In each experiment, the iv administration of endotoxin at the two doses of 400 ng/kg and 200 ng/kg led to increased respiration, intermittent cough and diarrhea, and a lack of alertness to surrounding. High fever (41 to 43°C) was recorded in all animals that lasted for 6 h. However, the general reactions to endotoxin was more pronounced at the dose of 400 ng/kg leading us to half-reduce the dose in Exp 2 and 3.
Exp 1: effect of endotoxin administration on jugular plasma levels of GH, ACTH, AVP, and cortisol

Endotoxin increased plasma GH concentration significantly (×5.9 ± 2.3) (P < 0.01) (Fig. 1). This sustained rise in GH concentration peaked 60 min after endotoxin administration. The hypothalamic-pituitary-adrenal axis was also rapidly and significantly activated (Fig. 1). This activation occurred earlier and was much more marked than that of GH. Mean plasma ACTH and cortisol levels increased 30 min after endotoxin administration with a maximum during the second hour (×21.4 ± 6.8 for ACTH and ×8 ± 1.9 for cortisol). They remained high during the first 5 (ACTH) and 6 (cortisol) h. Jugular AVP levels increased 30 min and peaked 45 min after endotoxin administration (×10.6 ± 3.1); this increase was rapid and transient, lasting for only 2 h.

Exp 2: effect of endotoxin administration on GH, jugular and portal SRIH, and portal GHRH plasma levels

Approximately 75 min after the endotoxin injection, we observed a significant increase in plasma GH levels (×6.2 ± 1.3) (P < 0.01) (Fig. 2). A second and major sustained increase in GH concentration was observed 4 h after endotoxin administration (×5.8 ± 0.6). After this second peak, plasma GH levels remained high and did not return to baseline until the end of the experiment. GHRH levels in portal plasma did not change significantly throughout the experiment (67.9 ± 4.3 pg/ml baseline vs. 70.7 ± 5.3 pg/ml after endotoxin). Its secretion was pulsatile, the pulsatility being observed particularly on individual profiles (Fig. 3). Approximately 45 min after the endotoxin injection, we observed a marked increase in SRIH levels both in jugular (×10.6 ± 2.4) and hypophysial portal blood (×7.9 ± 3) (Fig. 2). The increase in SRIH levels preceded slightly (15 min) the GH increase. The maximal jugular SRIH level was higher, although not significantly different to the maximal portal SRIH level (116.7 ± 15.4 pg/ml vs. 89.1 ± 33.2 pg/ml after endotoxin injection).

Exp 3: effect of consecutive administration of GHRH and endotoxin on GH and on jugular and portal SRIH

GHRH iv bolus induced as expected a significant increase in plasma GH levels (×18.4 ± 5.9) (P < 0.01) lasting 140 min and, in response to the following endotoxin administration, the jugular GH levels did not change significantly (4.7 ± 0.9 ng/ml in basal vs. 5.4 ± 0.5 ng/ml after endotoxin). But we observed a rapid and marked increase in jugular (×7.7 ± 0.6) and portal (×11.8 ± 4.5) SRIH levels after the endotoxin administration (Fig. 4). The maximal jugular SRIH level was slightly but not significantly higher than the maximal portal SRIH level (132.9 ± 39 pg/ml vs. 80.9 ± 15.4 pg/ml).

Discussion

We have shown that endotoxin induces a sustained increase of GH release in intact rams and these results are in agreement with the findings of Coleman et al. (17) in wethers. We observed as previously described an important stimulation of the hypothalamic-pituitary-adrenal (HPA) (7). This stimulation is more marked and precedes that of GH, suggesting as discussed below that CRH or cortisol secretion may be involved in the changes of GH secretion. A delayed and long-lasting increase of GH after endotoxin injection has also been recorded in human volunteers injected with low doses of LPS (1–4 ng/kg), the maximum ranging between 15 to 25 ng/ml and occurring 2–4 h after iv injection (15, 16, 33).
Surprisingly, in our experiment, the increase of GH was associated with an important rise of SRIH without a significant increase of GHRH. Our results concerning GHRH are different from the results obtained in rats. No study on GHRH secretion in portal blood has been performed in rats, but the in vitro results have shown an increase of GHRH release from hypothalamic explants (26) induced by IL-1β. Furthermore, LPS induced an increase in GHRH release from mediobasal hypothalamic (MBH) explants that was abolished by CRH antagonist and an interleukin receptor antagonist protein (IRAP) (34). The difference between rats and sheep may be due to the difference of approach, studies in vitro in rats and in vivo in sheep, or to the species difference.

For the first time, we report that endotoxin induces marked and significant rise in both hypothalamic portal and peripheral SRIH levels. This increase in portal SRIH is in agreement with a series of in vitro results obtained in rats (26, 34, 35). For instance, IL-1 stimulates SRIH release from acute hypothalamic explants and from diencephalic fetal cells in vitro (26, 34). On the other hand, the effect of IL-1 on SRIH release from mediobasal hypothalamic (MBH) explants is controversial (34, 35) but, in rats, the increase of SRIH has been correlated with a decrease of GH, the suppression of GH secretion by endotoxin being reversed by a pretreatment with an antiserum antisomatostatin (18). In sheep, no results are available on SRIH and GHRH regulation by endotoxin. It has just been shown that endotoxin induced IL-1 gene expression in choroid plexus (36).

The origin of portal SRIH remained to be determined. LPS could, directly or through the stimulation of cytokines synthesis, stimulate SRIH secretion by neurons of the periventricular nucleus (PeV) as shown in rats (26, 35). This effect might be subsequent to or only amplified by the activation of neurons synthesizing CRH. CRH has been shown to stimulate the release of SRIH in vitro (37, 38) and in vivo (39) (increasing SRIH secretion into hypothalamic portal blood). To determine if the portal SRIH increase is a consequence of an increased short feedback of GH, we studied the GH response to endotoxin animal whose GH pituitary pool has been depleted by a previous GHRH injection. Under that condition, despite the absence of increase of GH, SRIH levels were markedly increased after endotoxin treatment. Alternately, the increased levels of SRIH in HPB may simply reflect the contribution of peripheral SRIH to the level of SRIH in the hypothalamic-pituitary vasculature. So far, the rate of transfer of SRIH from peripheral circulation to long hypophysial portal has not been investigated.

For the first time, we have observed an experimental situation in sheep with a simultaneous and closed amplitude increase in jugular and portal SRIH levels. During another stressful conditions (a 5-min isolation contention), we have also observed an increase in portal SRIH levels, but no change in jugular SRIH was evidenced (40). An important peripheral SRIH increase has also been demonstrated in rats after endotoxin treatment (41) and in human during septic shock (Chayvialle, personal communication). In pigs, it has been shown that the increase of somatostatin was much higher in the abdominal portal vein than in the aorta or the internal jugular vein throughout the endotoxin infusion period (42). Peripheral SRIH probably arises mainly from the gastrointestinal tract as Taborsky and Ensinck (43) have shown that, in dogs, the pancreas is a minor source of circulating SRIH even when the D pancreatic cells are stimulated. Moreover, in dogs, TNFα has been shown to regulate somatostatin release from cultured fundic D cells (44). It has been hypothesized that peripheral somatostatin plays in rats an important role in the regulation of glucose levels during endotoxosis (41). It is well known that somatostatin induces a decrease in intestinal blood flow, capillary surface area, and intestinal consumption. Acting on smooth muscle of both arterioles and precapillary sphincters, it induces a potent...
vasoconstriction in the intestinal microcirculation (45, 46). During septic shock, severe evolution is associated with metabolic abnormalities, cardiovascular dysfunction, and multiple organ failure mainly related to ischaemia in part due to alterations in regional microcirculatory blood flow (47, 48). The gastrointestinal somatostatin rise could be a worsening factor in the evolution of multiple organ failure during septic shock.

The surprising association of a lack of change in GHRH release and an increased secretion of SRIH with the increase
of GH suggests that the effect of endotoxin on GH axis is mainly a pituitary one. In rats, a discordant effect of endotoxin at hypothalamic and pituitary levels has also been found: endotoxin induces an increase of GHRH secretion from hypothalamic explants (results obtained in vitro) contrasting with the in vivo decrease of GH. The important effect at the pituitary level has previously been shown in vitro. Indeed, Coleman et al. (17, 20) have shown that endotoxin stimulates GH release directly from ovine pituitary cultured cells. This increase of GH secretion is associated with an increased GH mRNA content and this effect is mediated through the lipoxygenase pathway.

The administration of LPS induces complex and multiple-step changes in the organism including a stimulation of cytokines release from peripheral macrophages, particularly a rapid increase of TNFα, IL-1, and IL-6. LPS and peripheral cytokines stimulate PGE-2 production by the central vasculature and regulate hypothalamic neurons (49). LPS as well as circulating cytokines stimulate both synthesis and secretion of pituitary IL-6 as well as they increase the expression of pituitary IL-1β and binding sites for cytokines. The effect of cytokines on pituitary cells has been extensively studied. In ovine pituitary cells, Nash et al. (21) have shown that TNFα increase both IL-6 and GH mRNA level, suggesting a paracrine/autocrine role for IL-6 in GH regulation. In rats, IL-6 stimulates GH release together with PRL and LH release.
from anterior pituitary cells (22). Furthermore, IL-1 receptors have been identified in rat pituitary and mainly on somatotrophs (50, 51). Moreover, endotoxin can have a physiological effect on pituitary cells since Abraham et al. (3) have established the presence of CD14 in cultured pig anterior pituitary cells.

In addition, LPS may increase the number of receptors for GHRH (GHRH-R) at the pituitary level. This effect may be secondary to the increased stimulation of the HPA axis. Indeed, glucocorticoids act directly at the pituitary level as potent stimulators of GHRH-R gene expression inducing a 5.6 ± 0.7-fold increase in GHRH-R mRNA levels (52). Finally, LPS could act on an unknown hypothalamic factor acting as a GH secretagogue. This factor might bind to the new GH-RP receptor that has been recently identified in hypothalamus and pituitary (53). It could explain the effect of endotoxin on GH without increase in GHRH secretion.

To better understand the mechanism of the prolonged effect of endotoxin on GH in sheep, it would be of interest to study its effect on GH receptor (GH-R) as well as on IGF-1 and insulin-like growth factor-binding protein (IGF-BP) levels. Indeed, a decrease of IGF-1 could explain the sustained effect of endotoxin on GH. In addition, in rats, IL-1β and TNFα to a lesser extent blunt the IGF-1 mRNA response to GH in hepatocyte primary culture. IL-1β decreases GH-R and GH-binding protein (GH-BP) mRNA levels (54, 55). Similarly, in steers, a reduction in plasma IGF-1 and IGF-BP2 and GH-binding protein (GH-BP) mRNA levels (54, 55). Similarly, in steers, a reduction in plasma IGF-1 and IGF-BP2 mRNA levels (54, 55).

Ultimately, our results show that endotoxin induces a complex reaction at multiple levels with a specific increase in both portal and peripheral SRH levels. The selective blockade of somatostatin should be useful for a better knowledge of the role of SRH in the physiology of septic shock.

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