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Pituitary Portal Plasma Levels of Oxytocin during the Estrous Cycle, Lactation, and Hyperprolactinemia

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INTRODUCTION

Oxytocin (OT)-containing neurons originating in supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus were for years thought to be supplied exclusively to the posterior lobe of the pituitary gland and concerned primarily with milk letdown, parturition, and osmoregulation. Recently, new OT-containing fiber systems have been identified in the median eminence¹⁻⁴ and have been implicated in the control of prolactin (PRL) and adrenocorticotropin (ACTH) secretion from the anterior lobe of the pituitary gland.⁵⁻¹² This paper summarizes experimental findings that evaluate the hypophysiotropic function of median eminence OT by characterizing neuropeptide secretion into the blood of pituitary portal vessels under physiological conditions when the secretion of PRL is known to be altered.

OXYTOCIN ACTION ON PROLACTIN SECRETION FROM THE ANTERIOR PITUITARY GLAND

Exogenous OT administration is known to stimulate PRL secretion both *in vivo* and *in vitro*,⁵⁻⁹ Oxytocin receptors have been identified in the anterior pituitary gland. Two pharmacologically distinct types of OT receptors have been characterized in this gland. One, resembling uterine-type OT receptors in its pharmacological specificity, has been proposed to mediate the OT action on PRL secretion.¹³ The other apparently binds with vasopressin and mediates OT action of ACTH release.¹⁴ Estrogen, which is a potent PRL stimulator,¹⁵⁻¹⁷ has been shown to modulate uterine-type OT receptors in the anterior pituitary.¹³ The intracellular mechanism of OT action on PRL release has not been characterized.

OXYTOCINERGIC NEURONAL PROJECTION TO THE MEDIAN EMINENCE: SOURCE OF THE PITUITARY PORTAL PLASMA OXYTOCIN

Oxytocinergic nerve fibers have been identified both in the external and internal layers of the median eminence; however, the majority of the fibers is found in the internal layer of the median eminence.^{1-4,18-20} The neurites in the external zone are axon terminals that originate in the parvocellular cell bodies in the PVN. These oxytocinergic terminals are localized in the immediate vicinity of primary capillary plexus of the pituitary portal vascular system and appear to be a major source of the portal OT. The fibers in the internal layer of the median

eminence are preterminal axons of magnocellular neurons from the PVN and SON in passage to the posterior pituitary gland. Recently published observations suggest that a proportion of portal OT may also be derived from these preterminal magnocellular axons in the internal layer of the median eminence.^{21,22}

CONCENTRATION OF OXYTOCIN IN PITUITARY PORTAL PLASMA: METHODOLOGICAL CONSIDERATION

A number of experimental paradigms are available for studying the hypothalamic regulation of PRL secretion from the lactotropes in the anterior pituitary gland. In view of the now-apparent multifactorial control of PRL release,^{23,24} measurement of hypothalamic hormones in pituitary portal blood appeared to be best suited for unraveling the complexities of neural regulation of PRL secretion. The major limitation of this approach in rats is that it requires acute anesthesia and surgical trauma. However, comparing pituitary portal plasma concentrations of hypothalamic hormones in rats under acute preparation with anesthesia with those of conscious sheep prepared with chronic cannulae, showed both preparations to have similar qualitative characteristics of the central regulatory process.²⁵⁻³² Thus, the measurement of hypothalamic hormone secretion using the portal preparation approach in rats appeared to provide physiologically relevant information on the central regulatory processes.

The choice of anesthetic has been shown to have impact on the hypothalamic hormone release into the blood of pituitary portal vessels. Alphaxalone (Saffan; Glaxo) anesthesia appeared to be best suited for studying the majority of neurohormone secretions.^{31,33-38} This anesthetic had very little effect on the elevated secretion of anterior pituitary luteinizing hormone (LH) and PRL before ovulation in rats.^{33,35} Under this anesthetic, a surge release of LH-releasing hormone (LHRH) into portal blood was observed before preovulatory gonadotropin release in female rats.³³⁻³⁴ In contrast, sodium pentobarbitone and urethane anesthesia blocked both gonadotropin and LHRH surges.³⁴ The level of thyrotropic-releasing hormone in pituitary portal plasma was higher under saffan than under either urethane or sodium pentobarbitone.³⁹ Urethane anesthesia appeared to abolish the circadian rhythm of ACTH, but not of corticosterone.⁴⁰ The level of somatostatin-14 in pituitary portal plasma was higher under urethane than under sodium pentobarbitone or Saffan.⁴¹

Our investigation on pituitary portal plasma concentrations of OT began with the determination of the anesthesia effect on the basal secretion of median eminence OT in ovariectomized rats. We used the transpharyngeal approach to expose the pituitary stalk^{31,42} and then collected pituitary portal plasma samples from these rats hourly for a period of two hours. The basal secretion of OT into the blood of pituitary portal vessels was similar in animals anesthetized with Saffan, urethane, or sodium pentobarbitone (Table 1). This study also revealed that OT is present in rat pituitary portal plasma in amounts much greater than those in peripheral plasma of ovariectomized rats. Using the transpharyngeal or parapharyngeal approach, several groups of investigators have also obtained 20- to 50-fold higher concentrations of OT in pituitary portal plasma than in peripheral plasma in male and female rats.^{22,40,43-45} It is unlikely that a significant amount of OT in pituitary portal plasma is due to backflow from the posterior pituitary gland, since the level of pituitary portal OT

did not change following removal of the pituitary gland (Table 1). It has been shown that a significant amount of OT is present in the cerebrospinal fluid (CSF) and in tanycytes lining the floor of the third ventricle.^{46–48} However, OT levels in the CSF are too low to significantly contribute to the relatively high OT levels in pituitary portal blood. Horn *et al.* have recently presented evidence that OT from the cut axons of the supraoptico-hypophysial tract does not significantly contribute to the high neuropeptide level in pituitary portal plasma following sectioning of the pituitary stalk.⁴⁴ Thus, the OT concentration in pituitary portal blood reflects the secretory activity of OT neurons in the median eminence.

CHANGES OF OXYTOCIN CONCENTRATION IN THE PITUITARY PORTAL PLASMA

Oxytocin Release during Reproductive Cycle

Gonadal steroids have long been known to influence OT levels in the hypothalamus and pituitary gland.^{49–57} Furthermore, recent studies have provided evidence of a gonadal steroid modulatory role on OT synthesis in the hypothalamus and of gonadal steroid induction of OT receptors in the anterior pituitary (see chapters by Brooks, Johnson, Pfaff *et al.*, and Schumacher *et al.*).¹³ An important consideration is whether or not the OT secretory activity in the median eminence alters with the dynamic changes of the plasma level of gonadal steroids during the reproductive cycle. To address this issue, we determined the concentration of OT in pituitary portal plasma during the estrous cycle in female rats under Saffan anesthesia. On each afternoon of the four-day estrous cycle, animals were anesthetized with Saffan, pituitary stalk was exposed, pituitary was removed, and pituitary portal blood samples were collected at one-hour intervals. The OT concentration in pituitary portal plasma collected between 1400 and 1600 hr of proestrus (1079 ± 185 pg/ml; $n = 12$) was significantly higher than that of any other time studied.⁵⁸ The mean OT concentration between 1600 and 1800 hr of proestrus was 494 ± 107 pg/ml ($n = 10$). There were no appreciable differences between the mean OT levels in portal blood obtained on the afternoon (1500–1700 hr) of estrus (198 ± 29 pg/ml; $n = 6$), diestrus-1 (283 ± 55 pg/ml; $n = 5$), or diestrus-2 (266 ± 49 pg/ml; $n = 6$). The OT concentration in peripheral plasma was low (58 ± 3 pg/ml; $n = 4$) throughout the cycle. Comparison of the pituitary portal plasma OT with peripheral plasma PRL in Saffan-anesthetized rats⁵⁸ indicated a positive correlation ($r = 0.828$; $p < 0.05$) between these hormones during the estrous cycle.

The elevated median eminence OT secretion into the blood of pituitary portal vessels immediately before the PRL surge during the afternoon of proestrus suggests a possible involvement of OT in preovulatory PRL release. This possibility is experimentally verified by studying the effect of immunoneutralization of endogenous OT with anti-OT serum in cyclic female rats (see also below).⁵⁹ A specific antiserum for OT was administered in female rats on the early afternoon (1330–1400 hr) of proestrus, and pituitary portal plasma samples were collected at one-hour intervals between 1400 and 1700 hr. Portal plasma samples were assayed for OT by radioimmunoassay after preincubation with *S. aureus* cells to adsorb IgG. This was followed by removal of this *S. aureus* cell-attached IgG from the pituitary portal samples using ODS silica disposable extraction columns. The OT level in pituitary portal plasma following OT antiserum treatment was very low (56–85 pg/ml) and

was 15- to 20-fold lower than those after the treatment with normal rabbit serum (NRS). It appeared, therefore, that the antiserum effectively neutralized the endogenous levels of OT in pituitary portal plasma during the afternoon of proestrus. Animals treated with OT antiserum showed attenuated elevation in systemic plasma PRL concentrations between 1500 and 1800 hr when compared to those in NRS-treated controls (Fig. 1). These data are consistent with the findings that passive immunoneutralization of endogenous OT significantly reduced, but did not completely abolish, the estrogen-induced PRL surge in ovariectomized rats.⁹

These results also agree with the finding that OT antagonist analogues prevented the preovulatory PRL surge in cyclic female rats.⁶⁰ Together, these data provide strong evidence for a physiological role of hypothalamic OT in the cyclic PRL secretion. However, it should be noted that passive immunoneutralization of endogenous OT failed to completely abolish PRL secretion. It is therefore possible that a multiplicity of factors is responsible for the integrated control of preovulatory PRL release. For example, a reduction of dopamine inhibition of PRL release may play a role, in part, in the rise in PRL secretion during proestrus.^{61,62} In addition to dopamine withdrawal, an increased secretion of hypothalamic vasoactive intestinal peptide (VIP) by elevated estrogen may also contribute to the preovulatory increase in PRL release.⁶³ Therefore, OT, dopamine (DA), and VIP appeared to be the major factors involved in multifactorial regulation of PRL release at the anterior pituitary level during the reproductive cycle.

Oxytocin Release during Lactation

The relative importance of the multiple releasing and inhibiting factors that modulate PRL secretion appears to vary according to the animal's reproductive status.^{23,24} Although it has been postulated that a putative releasing factor from the posterior pituitary may be essential for regulation of PRL secretion during lactation,⁶⁴⁻⁶⁸ hypothalamic PRL-releasing factors (PRFs) have also been implicated in modulation of suckling-induced PRL release.^{9,63} Since hypothalamic OT appeared to be a potential candidate of PRFs involved in suckling-induced PRL release, the OT level changes in pituitary portal plasma during lactation were determined. Portal plasma concentrations of VIP were also measured in order to compare the relative contributions of VIP and OT in the regulation of PRL release during lactation. A physiological role for hypothalamic VIP in suckling-induced PRL release has been proposed.⁶³

Pituitary portal plasma samples were obtained from 5 to 8-day postpartum lactating rats under urethane anesthesia. This anesthetic was chosen because, unlike Saffan and sodium pentobarbital anesthesia, urethane does not block suckling-induced PRL release (unpublished observation). Ten milliliters of 0.9% saline was injected before urethane anesthesia, since this treatment is known to prevent dehydration associated with urethane. The experiment was begun three hours after induction of anesthesia. All but one pup were removed from lactating mother rats six to twelve hours before experiment. In one set of rats, atrial catheters were implanted, and blood samples were collected at 15-min intervals for three hours. Six pups were reintroduced to the mothers 45 min after the beginning of blood sampling, and the pups were allowed to suckle for 135 min. Suckling was confirmed by

increased pup weight. Control mothers did not receive pups during the blood collection period. The plasma samples were measured for PRL.

In another set of animals, the pituitary stalk was surgically exposed, animals hypophysectomized, and pituitary portal plasma samples were collected at 45-min intervals. After the initial control sample, the response to suckling or control was examined, and samples were measured for OT and VIP. At the end of systemic or pituitary portal blood collection, suckled and control mothers were sacrificed, and each animal's mediobasal hypothalamus (MBH) was removed and measured for OT and VIP. Results indicated that PRL level rose significantly at 45 min after initiation of suckling (Fig. 2). Thereafter, levels continued to rise throughout the collection period and reached the peak at 135 min after pup introduction. However, in control animals, levels of PRL remained low throughout the collection period. Portal OT concentration was significantly increased during 0–45 min of suckling (Fig. 3), but the OT level did not change significantly after this period. The VIP level in pituitary portal plasma, however, gradually increased throughout the suckling period.

There was also a positive correlation between pituitary portal plasma VIP and systemic plasma PRL during suckling. OT and VIP concentrations in the MBH also responded to suckling. Hypothalamic OT decreased markedly in response to suckling in animals with intact pituitaries and decreased moderately in response to suckling in animals without the *in situ* pituitaries, (Fig. 4). Hypothalamic VIP also reduced in response to suckling in both pituitary intact and hypophysectomized mothers. Interestingly, however, the MBH levels of VIP in hypophysectomized suckled and nonsuckled mothers were significantly higher than those in suckled and nonsuckled mothers with *in situ* pituitaries. The positive correlation between VIP and PRL secretion during suckling and depletion of MBH VIP in the suckled mothers suggests that hypothalamic VIP can be a significant PRF during suckling-induced PRL release. This concept is further supported by the observation that passive immunoneutralization of VIP, with a specific antiserum for VIP, significantly blunted the suckling-induced PRL release.⁶³ It should be noted that MBH concentrations of VIP in both suckled and nonsuckled mothers were increased after removal of the *in situ* pituitaries, suggesting that an inhibitory pituitary factor is involved in the control of VIP secretion from the hypothalamus. These findings are in agreement with our recent data demonstrating the existence of a negative short-loop feedback action of PRL on hypothalamic VIP release (see below).⁶⁹

The brief increase in pituitary portal plasma OT levels, which also coincide with the initial rise of PRL, along with the decrease in MBH OT concentrations support the hypothesis that OT may modulate PRL secretion during lactation. Recently, Samson *et al.* have shown that infusion of OT antiserum into lactating female rats before pup reinstatement reduced subsequent PRL surges.⁹ These data are in agreement with the above view. However, this hypothesis should be viewed with caution because surgical removal of pituitary neurointermediate lobes (which would contain OT neuronal terminals) reduces the magnitude of the suckling-induced PRL surge⁶⁶ and because the possibility remains that the effect of OT antiserum on PRL release is the consequence of its effect on intermediate lobe OT.

It is noteworthy that the MBH concentration of OT responded less to the suckling stimulus in the hypophysectomized rats than in the rat with *in situ* pituitaries. This is perhaps indicative that a positive-feedback action of PRL (see below)⁶⁹ may also be involved in the interaction between MBH OT and pituitary PRL during lactation.

Oxytocin Release during Hyperprolactinemia

As noted above, there is a possibility of a bidirectional interaction between MBH OT and pituitary PRL in the regulation of PRL secretion. In order to further explore this possibility, we have determined the effect of hyperprolactinemia on OT secretion into the blood of pituitary portal vessels. Hyperprolactinemia was induced in ovariectomized rats by transplanting four anterior pituitaries to the kidney capsule for two to three weeks. Pituitary transplants induced hyperprolactinemia in these rats, since pituitary-transplanted rats showed elevated plasma PRL levels (3.8-fold) and decreased *in situ* pituitary weight (0.62-fold) and PRL concentrations (0.63-fold) as compared to the sham-transplanted rats.⁶⁹ Hyperprolactinemic rats showed a significant increase in the OT level in pituitary portal plasma (Fig. 5), indicating a stimulatory action of PRL on OT release from the hypothalamus. This stimulatory action of PRL on OT appeared to be unique and was not evident on the other hypothalamic regulators of PRL (dopamine and VIP). Hyperprolactinemic rats showed reduced DA and VIP levels in pituitary portal plasma, as compared to controls (Fig. 5). The observed stimulatory action of PRL on pituitary portal OT is consistent with our findings that PRL acutely increased OT release from the fetal hypothalamic neurons in primary cultures.⁶⁹ These results provide correlative evidence for the involvement of multiple mediators in the short-loop feedback action of PRL on PRL release. The increase in the secretion of OT following hyperprolactinemia is somewhat surprising in view of the PRL-releasing activity of OT (see above).

A positive-feedback system for OT has been demonstrated during parturition where cervical stretch by the fetus provides a feedforward system for further OT release to stimulate additional uterine contraction.⁷⁰ Hence, it may be possible that PRL secretion represents the operation of yet another feedforward system for OT secretion in order to further release PRL during lactation. Such a mechanism would not only facilitate OT-induced milk let-down, but would stimulate further PRL secretion to increase milk synthesis in preparation for the next feeding. The stimulatory influence of PRL on OT secretion may also explain the increased, possibly OT-mediated, maternal behavior during pregnancy and lactation.^{71,72}

SUMMARY

The median eminence receives fibers from both parvocellular and magnocellular OT neurons in the hypothalamus. The OT neuronal terminal in the median eminence is secretory and is the major source of the neuropeptide in the blood of pituitary portal vessels. The OT secretion into pituitary portal plasma increases by ovarian steroids, PRL, and the suckling stimulus. The OT secretion into the blood of pituitary portal vessels changes in parallel with the altered secretion of PRL from the pituitary. Because of correlative association between pituitary portal plasma OT and systemic plasma PRL and much abundant evidence for a direct stimulatory action of OT on PRL release, we propose that the pituitary portal vascular

system serves as the window for the central OT neurotransmission to the pituitary lactotropes.

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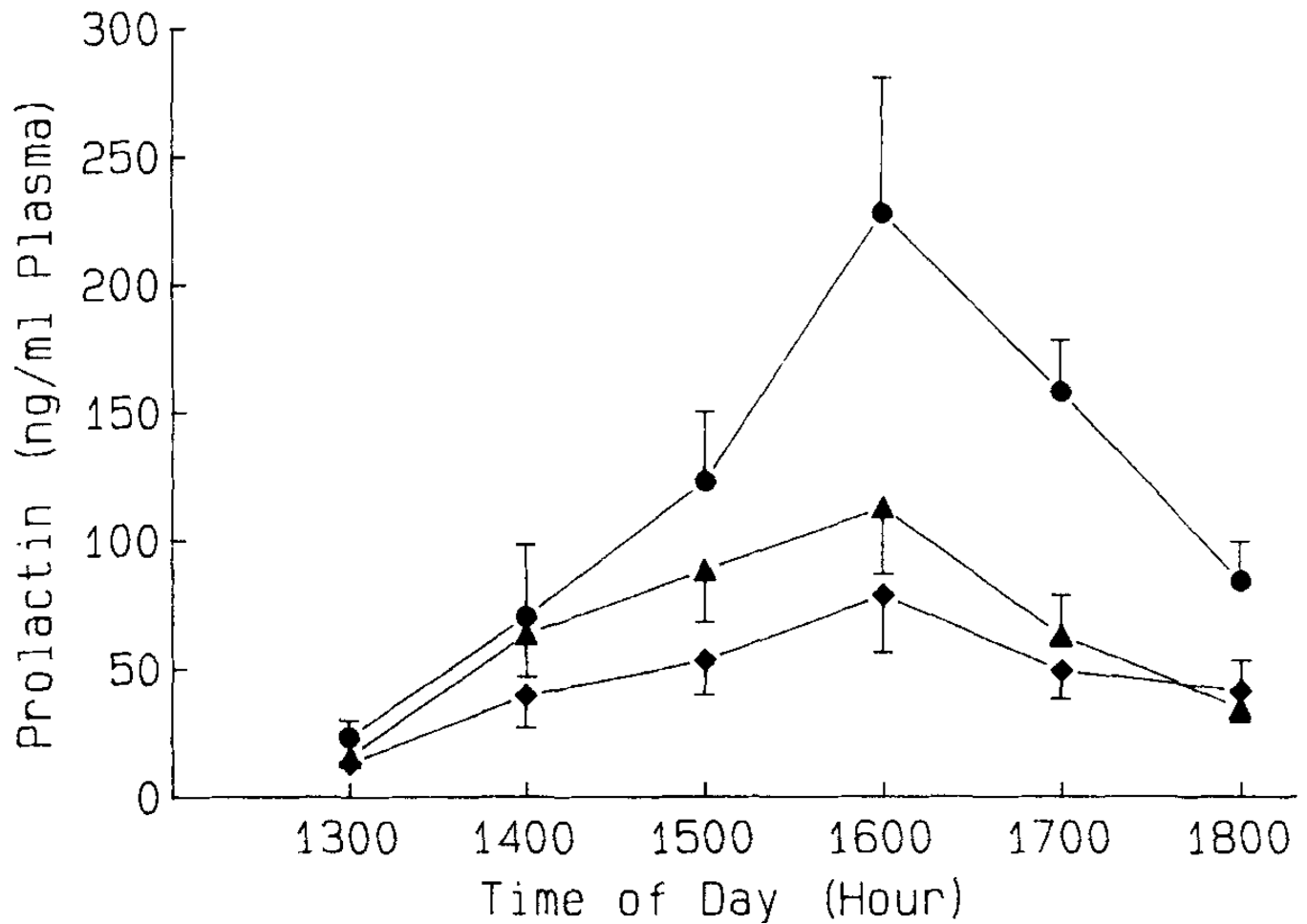


FIGURE 1.

Effect of an i.v. injection of normal rabbit serum (NRS; 0.25 ml; ●—●), NRS (0.15 ml) + anti-OT serum (AOTS; 0.01 ml; ▲—▲) or AOTS (0.25 ml; ◆—◆) on plasma PRL during the afternoon of proestrus in cyclic female rats. Values are mean \pm SEM of five to seven rats. (Reprinted from the original manuscript [Sarkar⁵⁹] with the permission of the S. Karger AG.)

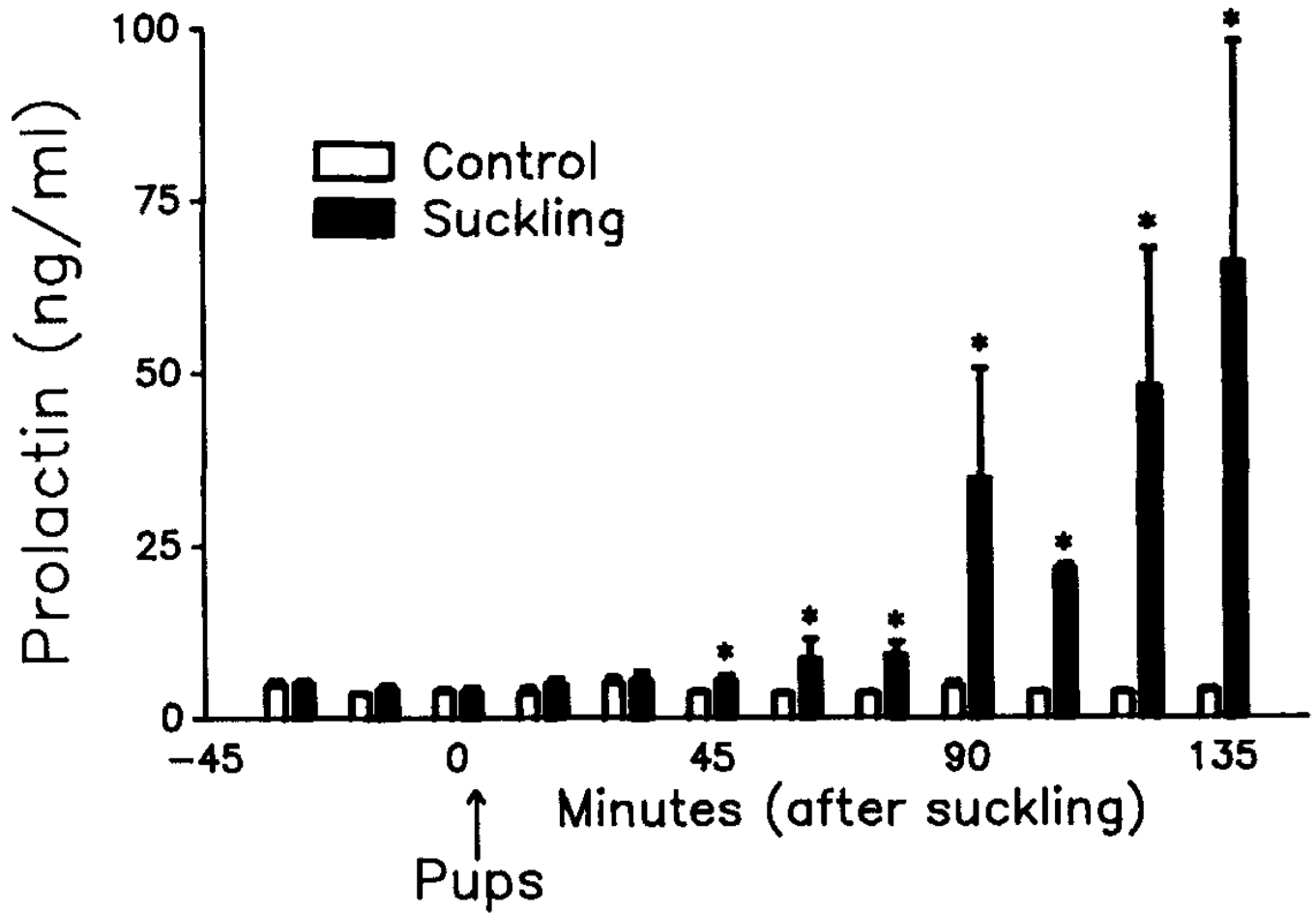


FIGURE 2.

Effect of suckling on PRL secretion in urethane (1 g/kg) anesthetized lactating rats. Values are the mean \pm SEM of five to six rats. *, $p < 0.05$.

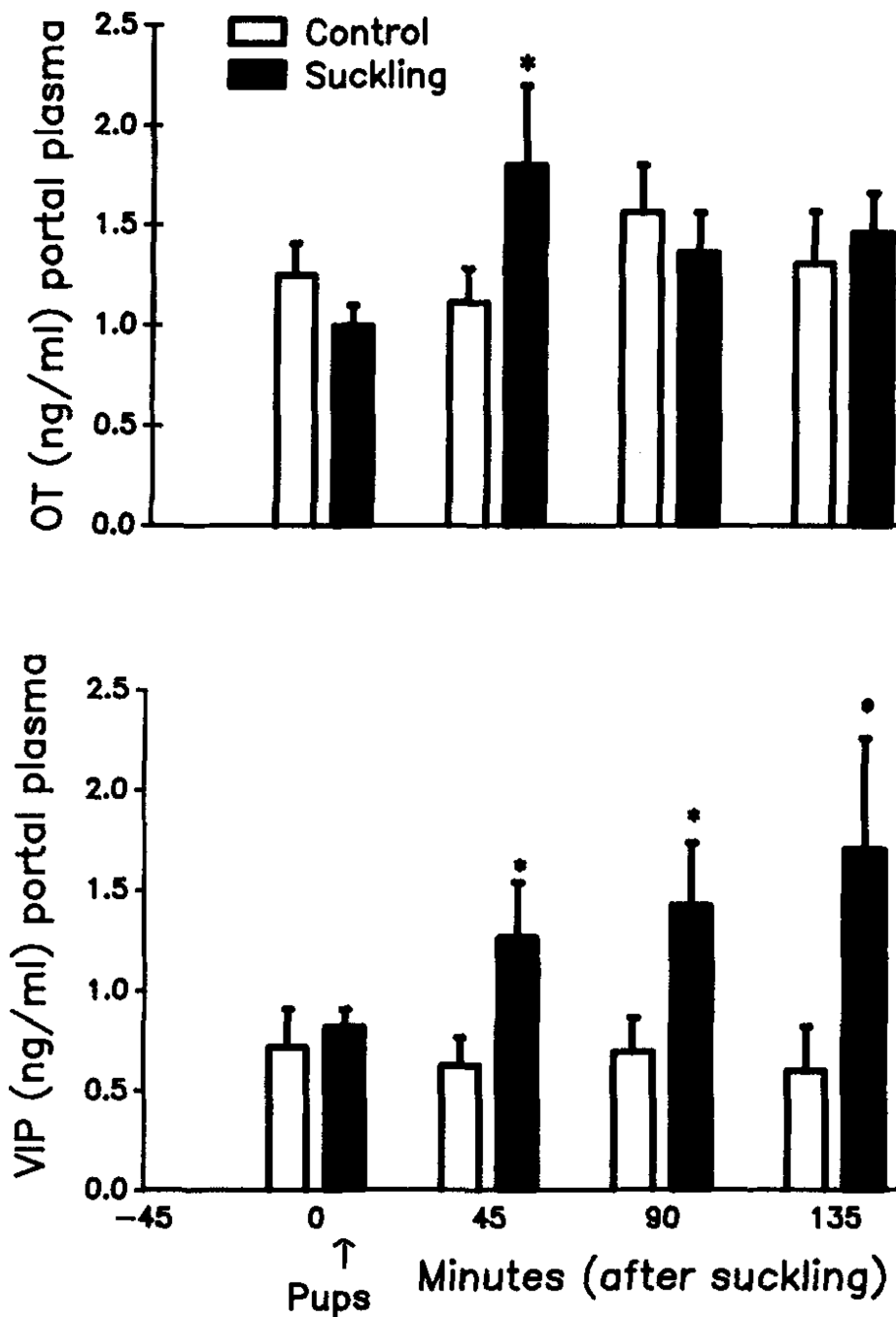


FIGURE 3. Effect of suckling on the concentrations of pituitary portal plasma OT (*upper panel*) and VIP (*lower panel*) in lactating rats under urethane (1 g/kg) anesthesia. Values are the mean \pm SEM of eight to ten rats. *, $p < 0.05$.

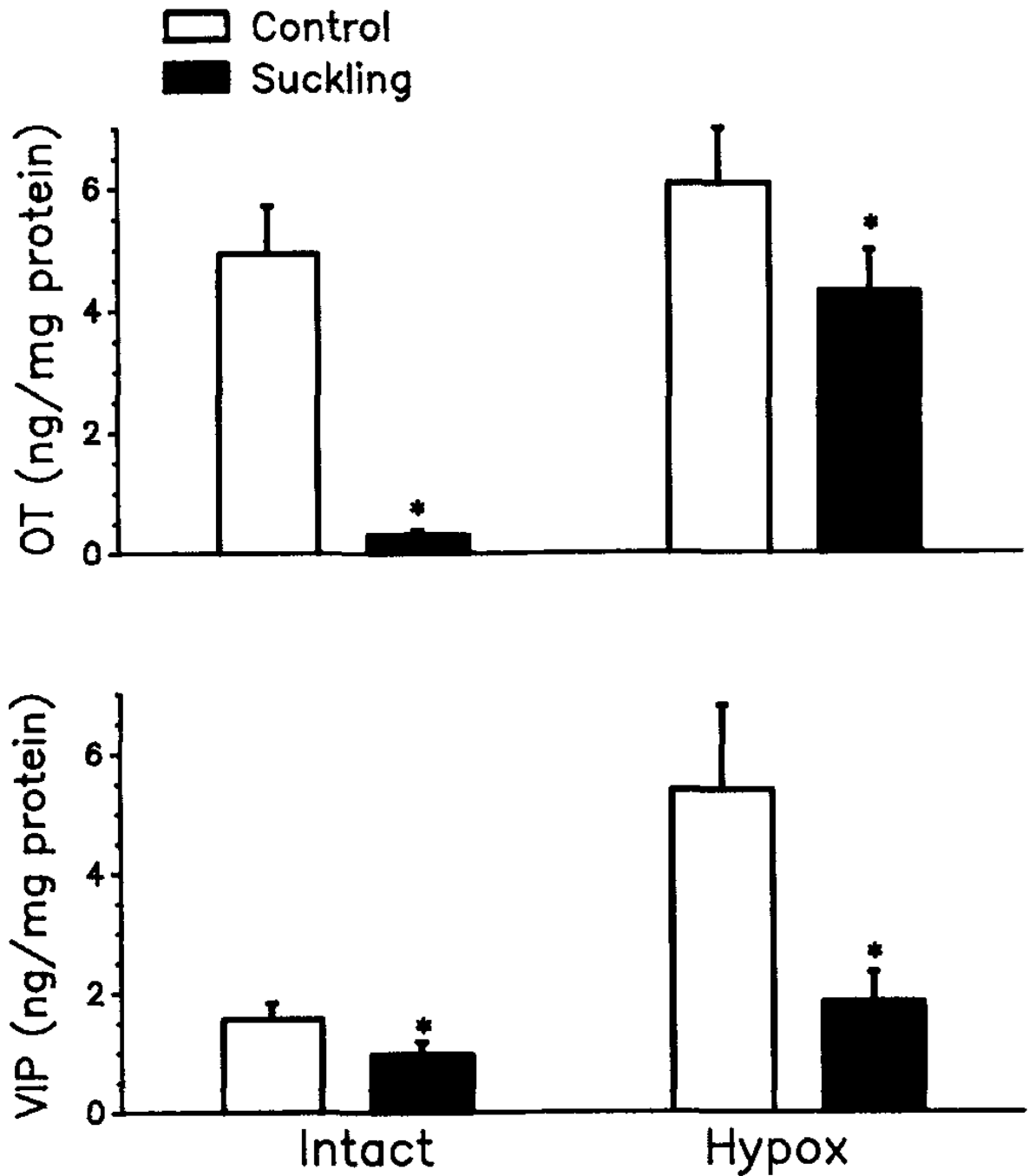


FIGURE 4.

Mean \pm SEM concentrations of OT (*upper panel*) and VIP (*lower panel*) in the mediobasal hypothalamus following suckling for 135 min in urethane-anesthetized lactating rats with intact *in situ* pituitary (Intact) or with pituitary removed (Hypox). Control intact rats and hypox rats were anesthetized with urethane and were deprived with pups: $n = 5-10$. *, $p < 0.05$.

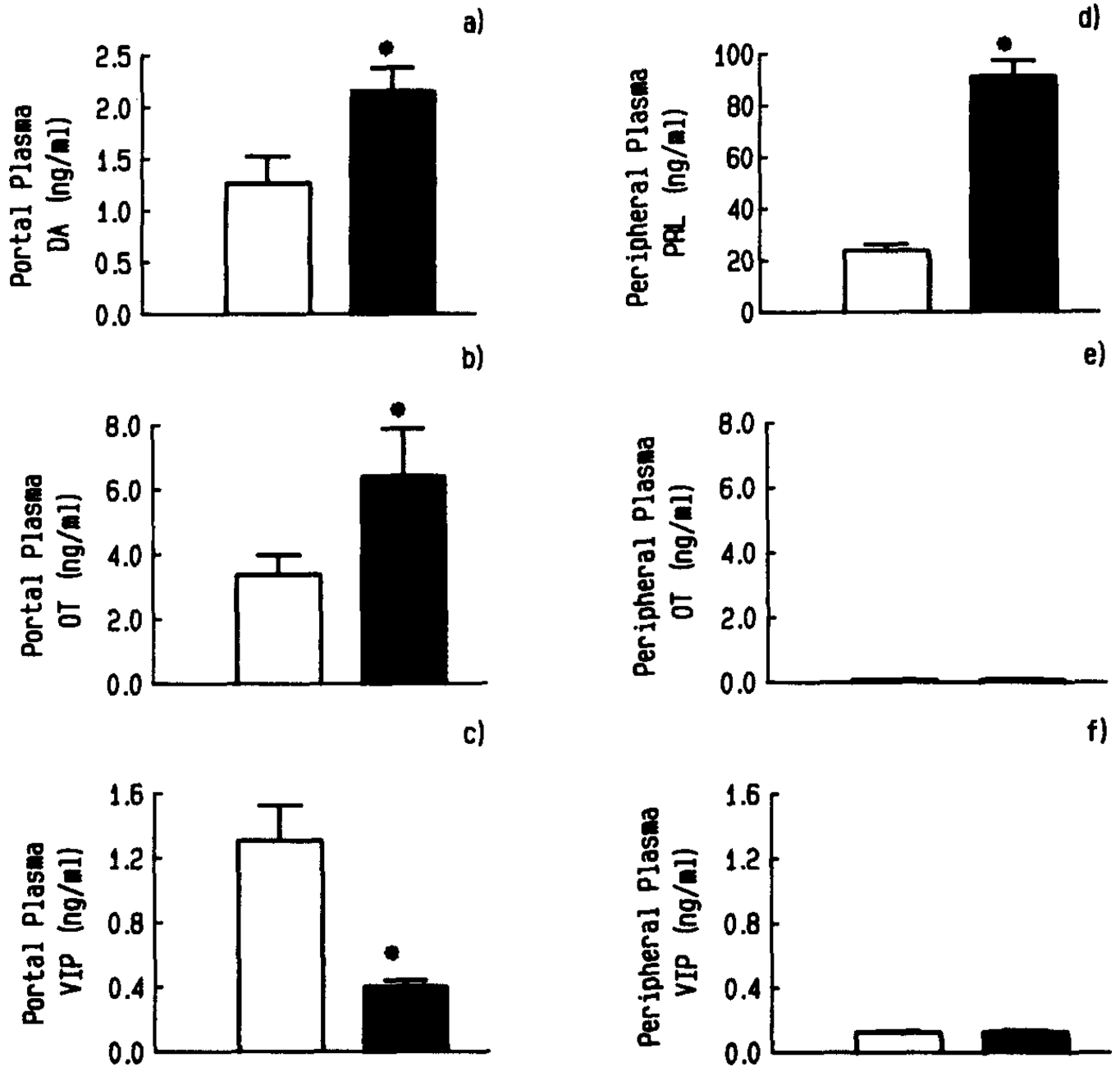


FIGURE 5.

Effect of hyperprolactinemia on the concentration of DA (a), OT (b), and VIP (c), in pituitary portal plasma and on the level of PRL (d), OT (e), and VIP (f) in peripheral plasma of ovariectomized rats. Hyperprolactinemia was induced by transplanting four anterior pituitaries (APs) under the kidney capsule (■). Control rats were sham AP-transplanted (□). $n = 7-10$; *, $p < 0.05$, compared to control. (Reprinted from the original manuscript [Sarkar⁶⁹] with the permission of the S. Karger AG.)

TABLE 1

Effect of Anesthetics and Hypophysectomy on Mean (\pm SEM) Oxytocin (OT) Concentration in Pituitary Portal Plasma and Peripheral Plasma of Ovariectomized Female Rats^a

Treatment	Portal OT (ng/ml)	Peripheral OT (ng/ml)
Saffan (Pituitary intact)	1.37 \pm 0.32	0.04 \pm 0.01 ^b
Saffan (Pituitary removed)	1.17 \pm 0.27	—
Urethane (Pituitary removed)	1.41 \pm 0.32	—
Sodium pentobarbital (Pituitary removed)	1.48 \pm 0.46	—

^a $n = 4-6$.

^b $p < 0.001$