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Lawrence E. Heisler[a]
Amanda J. Tumber[a]
Robert L. Reid[b]
Dean A. van Vugt[a,b]

[a] Department of Physiology, and
[b] Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Queen's University, Kingston, Canada

Vasopressin Mediates Hypoglycemia-Induced Inhibition of Luteinizing Hormone Secretion in the Ovariectomized Rhesus Monkey

Key Words
Stress
Gonadotropin
Vasopressin
Corticotropin-releasing hormone
Hypoglycemia
Rhesus monkey

Abstract
The objective of the present study was to examine the role of vasopressin in the regulation of LH secretion in the rhesus monkey. The effect of vasopressin administration on basal LH secretion and vasopressin antagonism on stress-induced inhibition of LH secretion were examined. Intracerebroventricular (i.c.v.) infusion of vasopressin (20 μg/h) to chair restrained ovariectomized rhesus monkeys (n = 5) decreased the area under the LH curve by −51.61 ± 13.73 ng/ml/h compared to −8.35 ± 7.11 ng/ml/h following infusion of artificial CSF (aCSF; p = 0.021). This effect was independent of any change in mean arterial pressure. Subsequently, the role of vasopressin in hypoglycemia-induced suppression of LH was examined. Administration of insulin (1 U/kg BW) to chair-restrained ovariectomized rhesus monkeys decreased the area under the LH curve by −60.88 ± 19.77 ng/ml/h. The decrease in LH was significantly different from that observed in aCSF-infused euglycemic controls which exhibited a slight decrease in LH (−8.35 ± 7.11 ng/ml/h; p = 0.036). In contrast, the area under the LH curve was increased slightly (1.42 ± 11.93 ng/ml/h) when insulin administration was combined with i.c.v. infusion of the vasopressin antagonist [deaminopenicillamine[1], O-methyl-tyrosine[2], arginine[8]]-vasopressin (120 μg/h; p = 0.013 vs. insulin only). The demonstration that vasopressin administration inhibits LH secretion whereas vasopressin antagonism prevents hypoglycemia-induced LH suppression suggests that vasopressin is a physiological inhibitor of LH secretion in the rhesus monkey.

Introduction

Insulin-induced hypoglycemia suppresses circulating LH levels in monkeys [1] and sheep [2], and inhibits hypothalamic multiunit activity associated with GnRH pulses in monkeys [3]. These observations are consistent with the suppressive effect of other experimental models of stress on LH secretion [4–6]. The effects of some stressors on the hypothalamic-pituitary-gonadal axis is thought to be mediated by endogenous opiates, inhibitory neuromodulators of LH and GnRH secretion [7]. Administration of naloxone, an opiate receptor antagonist, reverses the suppression of LH in response to many stresses [5, 8–10]. While naloxone prevented the hypoglycemia-induced suppression of LH in the ewe [2], we have shown it to be ineffective in reversing hypoglycemia-induced LH suppression in ovariectomized rhesus monkeys [1]. Furthermore, naloxone was unable to reverse the hypoglyce-

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Dr. Dean A. van Vugt
Department of Obstetrics and Gynecology
Etherington Hall
Queen's University
Kingston, Ont. K7L 3N6 (Canada)

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mia-induced inhibition of multi-unit activity in monkeys [3].

We subsequently postulated that hypoglycemia-induced inhibition of LH secretion is mediated by vasopressin. This hypothesis is derived from reports that hypoglycemia stimulates vasopressin secretion into the hypophysial portal blood of rats [11] and sheep [12, 13] and increases vasopressin depletion from nerve terminals in the median eminence [14, 15]. Furthermore, intracerebroventricular (i.c.v.) administration of vasopressin to rats [16] and monkeys [17] suppresses LH secretion and inhibits lordosis in rats [18], a GnRH-dependent behavior [19]. Histamine-induced stress does not inhibit LH levels in vasopressin-deficient Brattleboro rats, but significantly reduces LH levels in heterozygous controls [20]. Infusion of a vasopressin receptor antagonist into the cerebral ventricles of rhesus monkeys prevents the suppression of LH in response to interleukin-1α administration [21]. A direct effect of vasopressin on GnRH is suggested by the demonstration that vasopressin nerve terminals synapse on GnRH perikarya in the supraoptic nucleus of monkeys [22], and is further supported by the observation that vasopressin inhibits GnRH release from cultured GT_1 cells [23].

In the present study were investigated the ability of vasopressin to inhibit LH secretion in ovariectomized rhesus monkey. In addition, the role of vasopressin in hypoglycemia-induced suppression of LH was examined.

Materials and Methods

Animal Husbandry

All experiments were performed on 5 adult female rheus monkeys that had been ovariectomized at least 1 year prior to initiating this study. All monkeys were fitted with chronic indwelling cannulae in either the third (n = 2) or lateral ventricle (n = 3) at least 1 week prior to experiments as previously described [24]. The age of these monkeys ranged from 9 to 13 years and their weights were between 5 and 7 kg. All monkeys were housed in individual cages in light and temperature controlled rooms (lights on 06.00–18.00 h; temperature 20–22 °C). Their diet consisted of a twice a day ration of Purina monkey chow (Ralston-Purina, St. Louis, Mo., USA) which was supplemented with fruits and vegetables. Water was available ad libitum. All animal husbandry practices and all experimental procedures conformed to the guidelines of the Canadian Council on Animal Care and were approved by the Queen's University Animal Care Committee.

Experiment 1: Vasopressin on LH

Monkeys were lightly sedated with ketamine HCl (5–10 mg/kg; Rogarsetic, rogar/STB, Montreal, Quebec) and placed in primate restraint chairs between 08.00 and 09.00 h on the morning of each experiment. All monkeys had been acclimated to this form of restraint prior to the experiment. A cannula was inserted into the femoral vein for blood collection in order to monitor glucose and LH concentrations. Beginning at 10.00 h artificial cerebrospinal fluid (aCSF: 119 m*M* NaCl, 3.3 m*M* KCl, 1.3 m*M* $CaCl_2$, 1.2 m*M* MgCl, 1.2 m*M* Na_2HPO_4, 0.5 m*M* $NaHCO_3$, pH 7.4) was infused at a rate of 20 µl/h, and blood samples (2–3 ml) were collected at 15-min intervals for the duration of the experiment. Beginning 2–3 h later, aCSF infusion was continued or vaspressin (Sigma Chemical Co., St. Louis, Mo., USA) diluted to 1 µg/µl aCSF was infused for 3 h. In 3 of 9 experiments where vasopressin was administered, in addition to a femoral vein cannula for blood collection, a cannula was inserted into the femoral artery and connected to a P-1000B pressure transducer (Narco Biosystems, Houston, Tex., USA) in series with a MK-IV physiograph (Narco Biosystems) for measurement of blood pressure. Pressure was recorded for 5 min at 30-min intervals beginning 45 min prior to the vasopressin infusion. For comparison purposes, 2 monkeys received intravenous injections of vasopressin in a serial fashion. The doses of 0.1, 0.5, 1.0 and 5.0 µg were given at 45-min intervals in ascending order, and blood pressure was recorded for the first 5 min after each injection.

Experiment 2: Vasopressin Antagonism on Hypoglycemia-Induced LH Suppression

In a second series of experiments, monkeys were placed in primate chairs and prepared as described above. Blood samples were collected at 15-min intervals for 6–7 h beginning at 10.00 h. A bolus injection of 1.0 U insulin/kg BW (Humulin®, Eli Lilly, Toronto, Canada) or saline was administered into the femoral vein 2–3 h after initiating blood sampling. Infusion of aCSF (20 µg/h), begun at 10.00 h was either continued for the duration of the experiment or switched to the vasopressin antagonist, [deaminopenicillamine[1], O-methyl-tyrosine[2], arginine[8]]-vasopressin (dPTyr(Me)AVP) (Bachem California, Torrance, Calif., USA) 30 min prior to insulin injection. The antagonist was infused for 3.5 h at the rate of 120 µg/20 µl aCSF/h. Food was withheld throughout the experimental period, but water was available ad libitum.

Assays

Glucose levels were monitored throughout the experiment using a glucometer (Accuchek Glucometer IIM, Boehringer Mannheim, Laval, Quebec, Canada). Blood for LH determinations was refrigerated overnight prior to centrifugation at 1,500 *g*. Serum was collected and frozen at −20 °C until assayed. LH concentrations were determined from duplicate aliquots (100 µl) by radioimmunoassay as previously described [1]. Assay sensitivity, defined as the concentration of 100-µl reference preparation resulting in a mean cpm that was 2 SD less than the mean cpm for three zero standards (maximum binding) was 22–31 ng/ml. The intraassay and interassay coefficients of variation determined from three quality control serum pools assayed in triplicate at three volumes each was 6.14 and 9.59%, respectively.

Data Analysis and Statistics

LH levels frequently declined during the first half of the control period. Therefore, a baseline LH value for each experiment was determined after LH levels had stabilized. This baseline was established by calculating the area under the LH curve for the 1-hour period prior to insulin administration or vasopressin infusion (12.30–13.30 h). Summary measures of the LH responses to the various treatments similarly were determined for the 3-hour period following insulin administration or initiation of vasopressin infusion. The

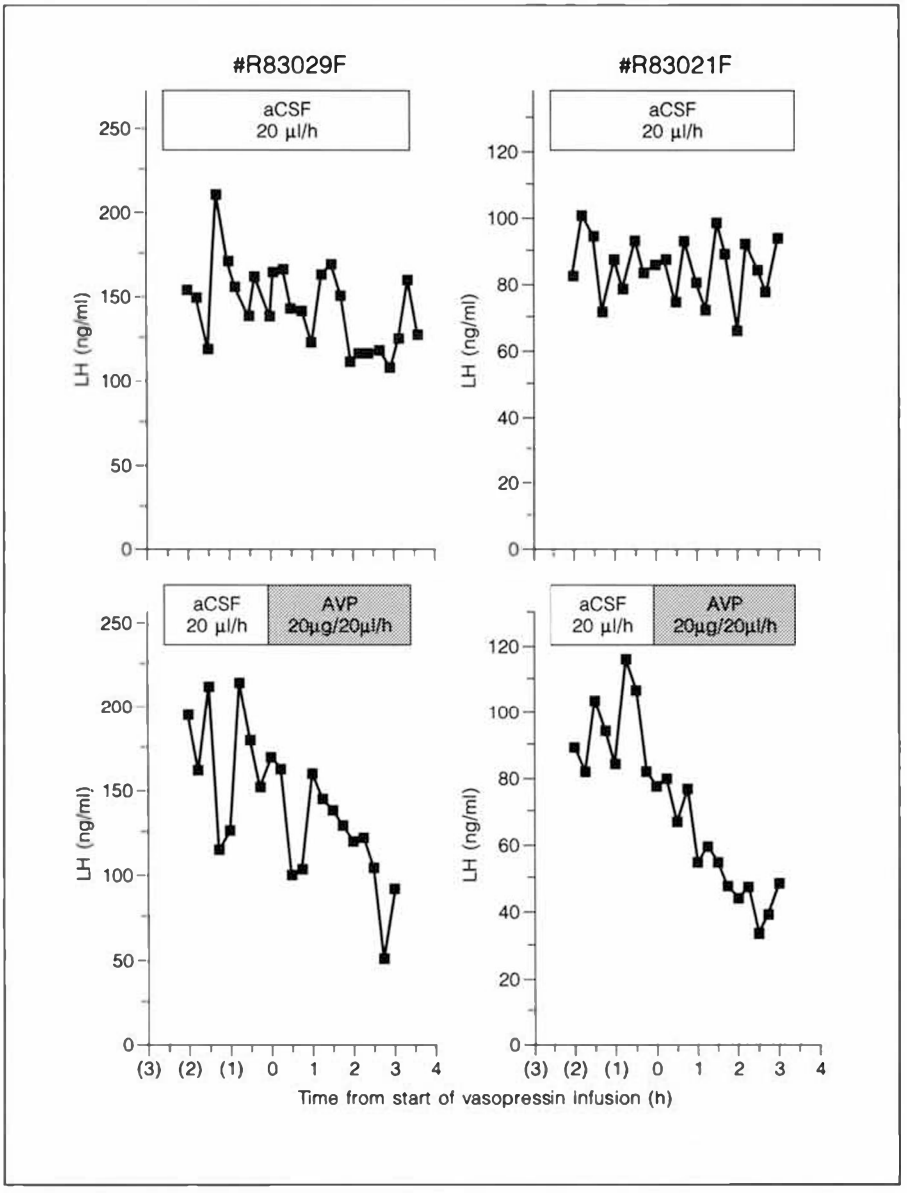

Fig. 1. Individual LH responses to vasopressin infusion. LH levels are shown for 2 chair-restrained ovariectomized monkeys during an infusion of aCSF (20 µl/h; top panel) or vasopressin (AVP; 20 µg/20 µl/h; bottom panel). Blood samples were taken at 15-min intervals. Vasopressin infusion was preceded by a 2- to 3-hour infusion of aCSF.

mean hourly response for the treatment period was subtracted from the 1-hour baseline LH value prior to calculating mean differences for each treatment group. Group means were compared by analysis of variance. Posthoc comparisons were made by Tukey's studentized range test using Systat (Evanston, Ill., USA). In addition, unpaired t tests were employed to determine differences where only 2 groups were compared. In all cases, significance was defined as $p < 0.05$.

Results

LH levels from 2 individual monkeys following i.c.v. infusion of aCSF or aCSF followed by vasopressin are presented in figure 1. Infusion of aCSF had no effect on LH concentrations whereas infusion of vasopressin resulted in a prompt suppression of LH. These experiments are summarized in figure 2 as the mean change in area under the LH curve. Vasopressin infusion resulted in a significant decrease in area under the LH curve compared to controls (-51.61 ± 13.73 vs. -8.35 ± 7.11 ng/ml/h; $p = 0.021$). Mean blood pressure calculated throughout the course of vasopressin infusions is presented in table 1. Vasopressin did not alter mean blood pressure at any time point during i.c.v. infusion as fluctuations above and below the pretreatment mean did not exceed 6%. This contrasts to the dose-related increase in blood pressure observed after intravenous administration of vasopressin.

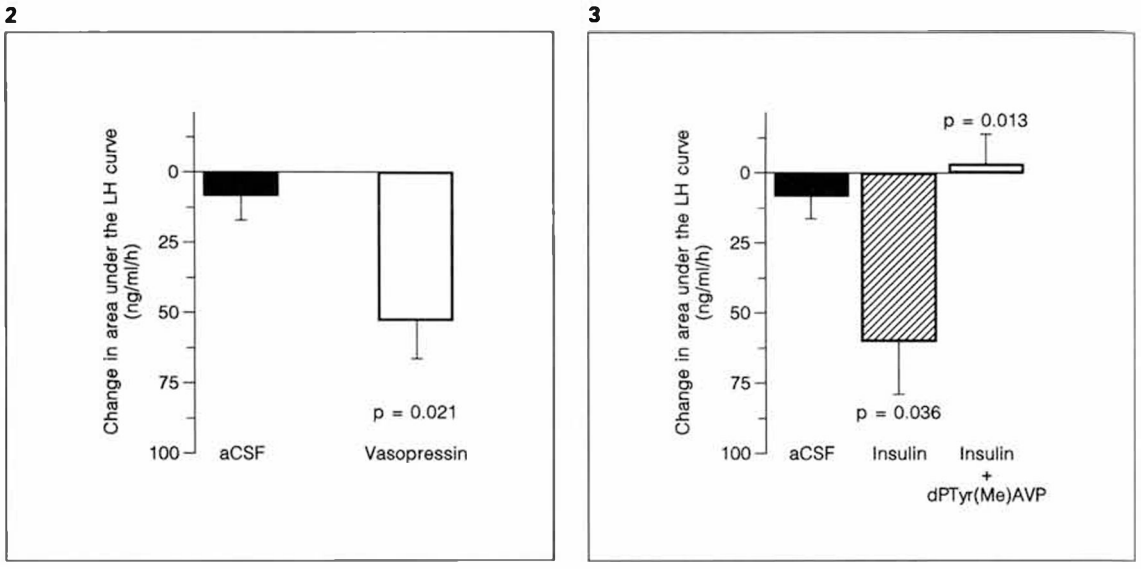

Fig. 2. Mean LH response to vasopressin infusion. The change in the area under the LH curve (AUC-LH) from the 1-hour baseline in response to infusion of aCSF at 20 µl/h (n = 5) or infusion of vasopressin at 20 µg/20 µl/h (n = 9) are compared. Vasopressin infusion resulted in a significant decrease in the AUC-LH (p = 0.021).

Fig. 3. Effect of vasopressin antagonism on the LH response to insulin. Compared are changes in the area under the LH curve (AUC-LH) from the 1-hour baseline in response to continued infusion of aCSF at 20 µl/h (n = 5), aCSF infusion plus 1 U/kg insulin (n = 5), or infusion of dPTyr(Me)AVP at 120 µg/µl/h plus 1 U/kg insulin (n = 5). Insulin resulted in a significant decrease in LH (p = 0.036) which was prevented by the vasopressin antagonist.

Table 1. Comparison of i.c.v. and i.v. vasopressin administration on mean blood pressure (mm Hg)

Route	Dose	Before	5 min	30 min	60 min	90 min	120 min	180 min	Maximum increase
i.c.v. infusion (n = 3)	20 µg/h	101 ± 4	99 ± 7	107 ± 1	103 ± 3	96 ± 6	99 ± 3	106 ± 6	6
i.v. bolus (n = 2)	saline	110 ± 3	109 ± 5						−1
	0.1 µg	112 ± 2	122 ± 1						10
	0.5 µg	101 ± 4	122 ± 4						21
	1.0 µg	111 ± 1	140 ± 2						29
	5.0 µg	107 ± 3	147 ± 2						40

Bolus injections of 0.1, 0.5, 1.0 and 5.0 µg vasopressin resulted in a progressive increase in mean blood pressure of 10% at 0.1 µg and 30% at 5 µg.

The LH responses to insulin (1 U/kg BW) with either continued infusion of aCSF or infusion of dPTyr(Me) AVP (120 µg/h) beginning 30 min prior to insulin are compared to the LH response in saline controls which received i.c.v. infusion of aCSF (fig. 3). The LH responses are expressed as mean decrease in area under the LH curve for each group. Administration of insulin resulted in a significant decrease in area under the LH curve compared to the saline-treated control group (−60.88 ± 19.77 vs. −8.35 ± 7.11 ng/ml/h, respectively; p = 0.036). Administration of dPTyr(Me)AVP prevented the decrease in the area under the LH curve in response to hypoglycemia (+1.42 ± 11.93 vs. −60.88 ng/ml/h; p = 0.013). Infusion of dPTyr(Me)AVP did not alter the degree of hypoglycemia produced by administration of insulin. Glucose levels fell from 5.24 ± 0.38 to 2.20 ± 0.11 mmol/l after insulin plus aCSF and from 5.20 ± 0.29 to 2.00 ± 0.28 mmol/l after insulin plus dPTyr(Me)AVP.

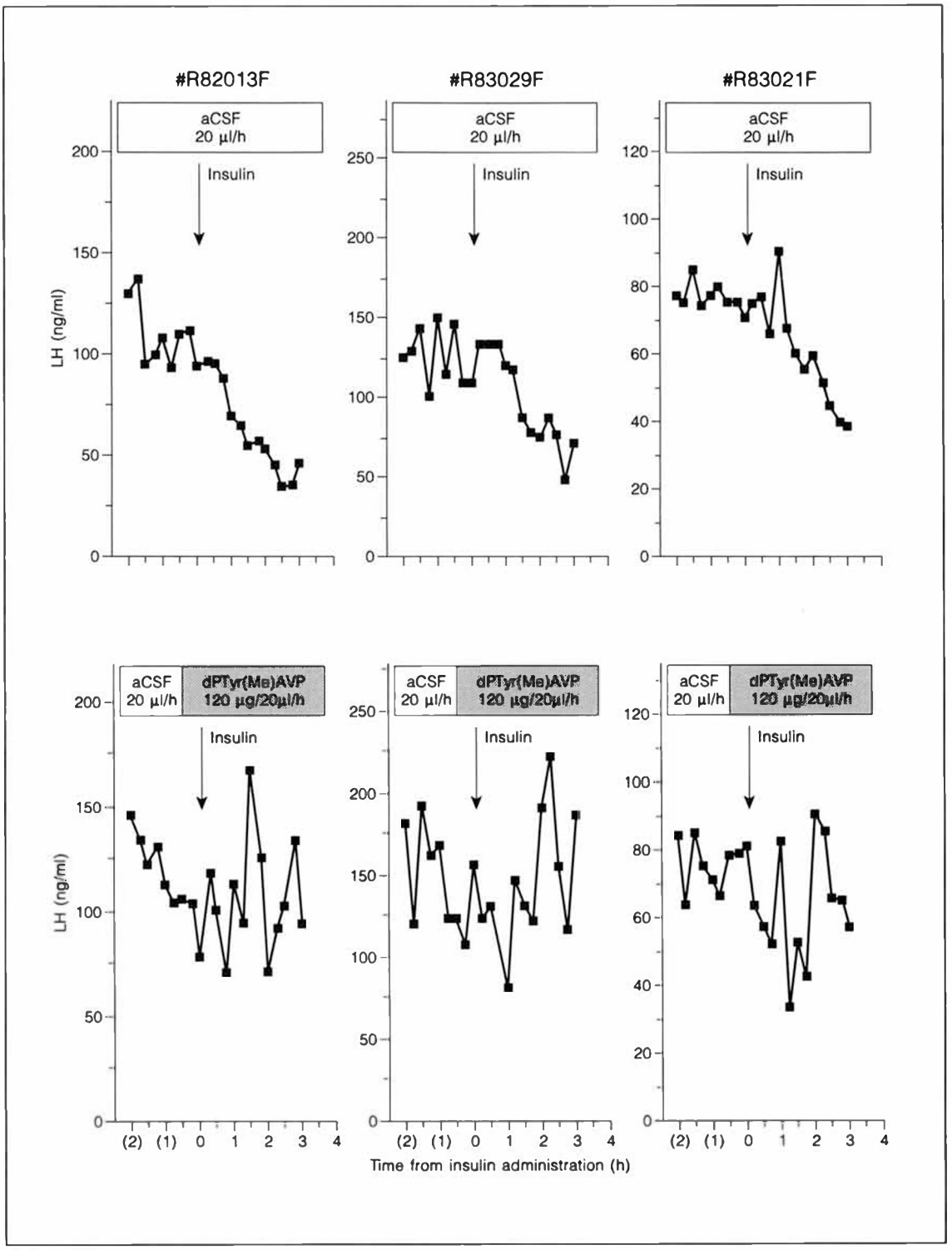

Fig. 4. Effect of dPTyr(Me)AVP on individual LH responses to insulin. LH levels are shown for 3 monkeys in response to insulin (1 U/kg) during an infusion of either aCSF (20 µl/h; top panel) or dPTyr(Me)AVP (120 µg/20 µl/h; bottom panel). dPTyr(Me)AVP infusion began 30 min prior to administration of insulin.

Figure 4 illustrates LH responses in 3 of the 5 monkeys administered insulin with continued infusion of either aCSF or infusion of dPTyr(Me)AVP. Insulin plus aCSF resulted in a gradual decline in LH levels. Nadir LH levels were usually observed between 2 and 3 h after insulin injection and were typically 50–60% of basal levels. The LH responses to insulin administration plus infusion of dPTyr(Me)AVP are shown for the same 3 monkeys. Hypoglycemia-induced suppression of LH was completely prevented in 2 of the 3 experiments shown. Fluctuations in LH levels were consistent with pulsatile release. LH levels in the third example declined acutely in response to insulin prior to returning to pretreatment levels midway through the treatment period. The 2 remaining animals for which the individual data are not shown had responses that were intermediate to the 3 depicted.

Discussion

This study confirms the previous observation that insulin-induced hypoglycemia suppresses LH in ovariectomized rhesus monkeys [1, 3]. We have demonstrated that i.c.v. infusion of dPTyr(Me)AVP reversed hypoglycemia-induced suppression of LH, suggesting a role for vasopressin in this model of stress. Furthermore, administration of vasopressin into the cerebral ventricle inhibited LH levels to the same degree observed in response to insulin-induced hypoglycemia.

The effect of vasopressin on LH secretion is presently controversial. Vasopressin administration has been observed to suppress LH when administered intracisternally to rats [16]. Furthermore, similar experiments to ours conducted in ovariectomized rhesus monkeys also showed a suppression of LH in response to vasopressin [17]. In contrast to these inhibitory effects, subcutaneous administration of vasopressin to baboons stimulated LH release [25]. In addition, intravenous administration of vasopressin to rats was able to stimulate LH release [26, 27], but prevented the estradiol-induced LH surge [26]. These contradictory responses to vasopressin may be explained by the mode of administration. Stimulation of LH in response to vasopressin administered intravenously or subcutaneously could be due to alterations in blood pressure [26, 27], or to a direct effect of vasopressin on the gonadotroph [28]. We examined blood pressure responses to vasopressin administration in several monkeys since increases in blood pressure were observed in rats after administration of high doses of vasopressin into the cerebral ventricle [29]. Since hemodynamic stimuli release corticotrophin-releasing hormone (CRH) from the paraventricular nucleus, an alteration in blood pressure might influence LH release [30]. However, we did not observe a change in blood pressure throughout the course of i.c.v. vasopressin infusion.

In addition to showing that exogenous vasopressin reduced LH secretion, our experiments suggest that inhibition of LH secretion in response to hypoglycemia is mediated by vasopressin. A variety of stressors, including hypoglycemia, have been shown to increase hypothalamic secretion of both CRH and vasopressin [11–14], and to increase vasopressin expression in CRH containing cells of the paraventricular nucleus [15]. Insulin-induced hypoglycemia stimulates vasopressin secretion from both hypophysiotropic [31] and neurohypophyseal vasopressin neurons [32, 33]. Therefore (1) inhibition of LH secretion by exogenous vasopressin, (2) stimulation of vasopressin secretion by hypoglycemia, and (3) prevention of hypoglycemia-induced suppression of LH by vasopressin antagonism, constitute compelling evidence for vasopressin being an inhibitory neuromodulator of LH secretion. This conclusion is substantiated by the report that vasopressin antagonism blocked interleukin-1α-induced suppression of LH in the rhesus monkey [21].

We speculate that vasopressin suppresses LH via an inhibitory effect on GnRH secretion. A direct effect is supported by the in vitro observation that vasopressin inhibits GnRH release from GT_1 cells [23]. Synaptic connections between vasopressin neurons and GnRH perikarya have been observed in the supraoptic nucleus of juvenile cynomolgus monkeys [22]. Furthermore, vasopressin neurons originating in the supraoptic nucleus and parvocellular region project to the zona externa where they may interact with GnRH terminals [14, 15, 34, 35]. An interaction between vasopressin and GnRH nerve terminals in the median eminence is consistent with the effectiveness of i.c.v. injection of either vasopressin or its antagonist since diffusion of these compounds from the ventricle to supraoptic GnRH perikarya is unlikely [36].

In addition to direct effects on GnRH release, vasopressin could stimulate inhibitory neuromodulators of GnRH. Vasopressin was reported to stimulate β-endorphin release from the hypothalamus [37, 38]. However, vasopressin stimulation of opioids does not appear to be the mechanism whereby hypoglycemia suppresses LH since naloxone was unable to block hypoglycemia-induced suppression of LH [1, 3]. However, it is feasible that an opioid pathway is activated by other stressors or by administration of vasopressin.

The vasopressin antagonist used in the present study preferentially binds to the V_1 receptor [39], the vasopressin receptor subtype identified in most brain tissue [40]. This antagonist also exhibits an antioxytocic effect although it is weaker than its antivasopressin effect [39]. This may be significant since oxytocin is stimulated by a variety of stresses including hypoglycemia [31, 33, 41]. While it is possible that dPTyr(Me)AVP binds to oxytocin receptors, it is not clear how antagonism of oxytocin would prevent hypoglycemia-induced suppression of LH since oxytocin is predominantly stimulatory to LH secretion [42].

In conclusion, we have demonstrated that intraventricular administration of vasopressin to ovariectomized rhesus monkeys inhibits LH secretion whereas antagonism of endogenous vasopressin prevents hypoglycemia-induced suppression of LH. These studies suggest a role for vasopressin as an inhibitory neuromodulator of LH secretion that may mediate the inhibitory effects of stress on reproductive function.

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