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The Effect of Dopamine Antagonists and/or VIP on TRH- or VIP-Induced Prolactin Release in Estrogen- and Progesterone-Treated Ovariectomized Rats[1] (42674)

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Abstract. These experiments were conducted to test the hypothesis that the effectiveness of VIP in releasing prolactin is, like TRH, enhanced when preceded by a short period of dopamine receptor antagonism. Chronically catheterized, ovariectomized rats pretreated with estradiol benzoate and progesterone to mimic early pregnancy were used throughout these studies. In the first experiment, animals were injected either with the dopamine (DA) antagonist domperidone (DOM, 0.01 mg/rat, iv) or with vehicle (acetic acid in saline). Five minutes later, all animals were treated with the DA agonist 2-Br-α-ergocryptine maleate (CB-154, 0.5 mg/rat, iv) followed 60 min later by the administration of thyrotropin-releasing hormone (TRH, 1.0 μg/rat iv) or vasoactive intestinal peptide (VIP, 25 μg/rat, iv). The injection of TRH following DOM treatment increased mean plasma PRL levels 100 ng/ml above levels found in vehicle-injected rats. VIP administration, however, increased PRL levels in the blood in DOM-treated rats only 6 ng/ml above the levels in vehicle-injected animals. The same treatment protocol was used in the second experiment except that the DA antagonist, sulpiride (0.01 mg/rat, iv) was administered instead of DOM, and CB-154 was not given. In this experiment both TRH and VIP released PRL. The response to TRH, but not to VIP, was significantly greater following sulpiride than in animals treated with sulpiride vehicle. In the third experiment animals were treated with DOM, VIP, DOM plus VIP, or vehicle. Five minutes later all rats received CB-154 injections, followed 60 min later by TRH administration. The final experiment was a replicate of the third except that sulpiride was substituted for domperidone and no CB-154 was given. The resulting data revealed that (1) dopamine antagonism enhanced the effectiveness of TRH but not VIP and (2) that VIP augmented the effectiveness of DA blockade on PRL release and was additive with domperidone (but not sulpiride) on increasing the responsivenss to TRH. However, VIP administration without concurrent administration of domperidone or sulpiride did not increase the effectiveness of TRH compared to vehicle-injected animals. From these data we concluded that VIP is a PRL-releasing hormone the effect of which is not affected by interruption in dopamine tone as is observed for TRH. Second, VIP can potentiate the stimulatory actions of at least one DA receptor antagonist and TRH on PRL release. This later finding suggests that VIP may play a modulatory role in the neuroendocrine regulation of PRL secretion in the female rat.

Vasoactive intestinal peptide (VIP) has been found in the hypothalamus (1), is released into the hypophysial portal circulation (2–4), and stimulates prolactin (PRL) release both *in vivo* and *in vitro* (5–8). In addition, the injection of anti-VIP serum into lactating rats inhibits suckling and ether stress-induced PRL release (9). These studies suggest that VIP is a physiological PRL-releasing factor (PRF).

Thyrotropin-releasing hormone (TRH) is also among the group of hypothalamic factors being considered as putative PRFs. This peptide has been shown to increase in the hypophysial portal blood during suckling (10, 11) and on the afternoon of proestrus (10). Further, it has been demonstrated that anti-TRH serum can suppress PRL release on the afternoon of proestrus (12, 13).

The PRL-releasing action of TRH is enhanced *in vivo* (11, 14) and *in vitro* (15) when preceded by a transient decrease in dopamine (DA) at the anterior pituitary (AP). In a recent series of experiments, we have confirmed that this augmentation of TRHs effectiveness by transient DA blockade is present during lactation (unpublished) and extended this observation to several days of

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the estrous cycle (unpublished), early pregnancy (16), and pseudopregnancy (17). Further, we have demonstrated in ovariectomized (OVX) rats that this secretory mechanism was induced by estrogen (E) and maintained and amplified by progesterone (P) (18). The purpose of the present study was to test the hypothesis that the effectiveness of VIP as a releasing hormone is enhanced like TRH by a transient decrease in dopamine at the lactotroph. In a previous study using lactating rats, VIP (1 μg/rat, iv) did not stimulate PRL release (unpublished observation). Therefore, in the present investigation we increased the dosage of VIP treatment to 25 μg/animal, iv, which is similar to maximal levels injected by other investigators (7). In addition, E- and P-treated OVX rats, which we found to be quite sensitive to TRH especially after DA antagonism (18), were used instead of lactating rats.

Materials and Methods. *Animal protocol.* Female Sprague–Dawley rats (250–275 g) were purchased from Holtzman Inc. (Madison, WI). They were housed two per cage in a room with controlled lighting (14 hr of light, 10 hr of darkness, lights on at 0600 hr), temperature (23°C), and humidity (50%). Food (Purina Rat Chow) and water were available at all times. Four to ten days after arrival, the animals were OVX under methoxyflurane (Pittman-Moore, Ft. Washington, PA) anesthesia. Steroid treatment began 8 to 10 days after OVX and blood samples were obtained after a 6-day steroid treatment schedule which we previously used to mimic Day 3 of pregnancy (18). Estradiol benzoate (EB) was injected subcutaneously at 0.5 μg/rat followed 17 hr later by 50 μg/rat in 0.2 ml sesame oil. This EB treatment protocol has been shown to be effective in stimulating a proestrous-like afternoon PRL surge in OVX rats (19). Progesterone was injected sc at two doses (0.5 and 1.0 mg/0.2 ml sesame oil), in the estrogen-primed rats in a schedule shown in Table I. The doses of progesterone were derived from previous studies in our laboratory (unpublished). A Silastic intraatrial catheter was implanted in each animal 3 days before the initiation of the experiment.

Drug treatment. The specific dopaminergic D_2 receptor antagonists, domperidone (Janssen Pharmaceutica, Piscataway, NJ) and sulpiride (Delangrange International, Paris, France), were dissolved in a small volume of 7% acetic acid and diluted in physiological saline to their final concentration of 0.01 mg/0.1 ml which was administered intravenously. The DA receptor agonist, 2-Br-α-ergocryptine maleate (CB-154; Sandoz Ltd., Basel, Switzerland), was dissolved in 50% ethanol and injected iv at the dose of 0.5 mg/rat. Vasoactive intestinal peptide and thyrotropin-releasing hormone (Sigma Chemical Co., St. Louis, MO) were dissolved in physiological saline and injected iv at the doses of 25 and 1.0 μg per rat, respectively.

Experimental protocols. At 0800 hr on the morning of the experiment, a polyethylene extension and stopcock were attached to the exteriorized portion of each animal's catheter. Blood sampling and drug treatment began at 0930 hr. Each blood sample (0.4 ml) was replaced by an equal volume of heparinized (50 U/ml) physiological saline. The plasma recovered was frozen at −20°C until assayed for PRL.

Experiment 1: Five minutes after the initial blood sample was obtained, domperidone or vehicle (acetic acid in saline) was

TABLE I. STEROID TREATMENT PROTOCOL IN RATS THAT WERE OVARIECTOMIZED (OVX) FOR 7 DAYS[a]

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
0.5 μg EB[b] (1600 hr)[c]	50 μg EB (0900 hr) 1.0 mg P (1700 hr)	No treatment	0.5 mg P[d] (0700 hr)	0.5 mg P (0700 hr) 1.0 mg P (1700 hr)	2.0 mg P (0700 hr) Sample (0930 hr)

[a] Steroids were injected subcutaneously in 0.2 ml sesame seed oil per injection.
[b] EB = estradiol benzoate.
[c] P = progesterone.
[d] Numbers in parentheses indicate the treatment or sample time.

administered iv. Five minutes after the administration of domperidone or vehicle, a second blood sample was obtained followed by an injection of CB-154. Additional blood samples were taken at 10, 30, and 60 min after CB-154. After the 60-min sample was obtained, TRH or VIP was injected iv and blood samples were taken 5, 10, 15, and 20 min later.

Experiment 2: Five minutes after the removal of a control blood sample, sulpiride or vehicle (acetic acid in saline) was administered to each animal. Additional blood samples were obtained 5, 15, 35, and 65 min after sulpiride or vehicle treatment. Following the sample taken at 70 min of the experiment either TRH or VIP was injected iv and blood samples were obtained 5, 10, 15, and 20 min later.

Experiment 3: An initial blood sample was obtained followed 5 min later by the iv administration of either domperidone, domperidone plus VIP, or vehicle (acetic acid in saline) plus VIP. A second blood sample was obtained 5 min later followed by an injection of CB-154. Additional blood samples were taken at 10, 30, and 60 min after CB-154. Following the sample taken at 70 min of the experiment, TRH was injected iv and blood samples were obtained 5, 10, 15, and 20 min later.

Experiment 4: Following the withdrawal of an initial blood sample, sulpiride (or its vehicle), VIP (or saline), or sulpiride and VIP were administered. Blood samples were withdrawn 10, 30, 60, and 90 min after these treatments. Immediately after the 90-min sample was drawn, TRH was administered with additional samples drawn 5, 10, 15, and 20 min after TRH treatment.

Prolactin radioimmunoassay. Plasma PRL was assayed using a specific rat PRL radioimmunoassay at two dilutions in duplicate with NIAMDD-RP-I-5 as the iodinated preparation and NIAMDD-RP-1 (11.0 IU/mg) as the standard (20).

Statistical analysis. The data from the two treatment groups were compared at each time interval by the F test. Values which were different at the $P < 0.05$ level were considered to be significant.

Results. The effect on PRL release of transient DA blockade with domperidone in OVX–steroid-treated rats is shown in Fig. 1. The administration of domperidone resulted in a significant increase in plasma PRL within 5 min compared to vehicle-treated animals. The injection of the dopamine agonist CB-154 lowered plasma PRL levels in domperidone-treated rats such that within 60 min PRL levels were not significantly different from vehicle-treated control rats. The administration of TRH (Fig. 1A) stimulated PRL release in both groups; however, mean PRL levels in the blood increased to 248 ng/ml in domperidone-treated animals compared to 148 ng/ml in vehicle-treated control animals wthin 5 min. The difference was statistically significant. Figure 1B shows that the

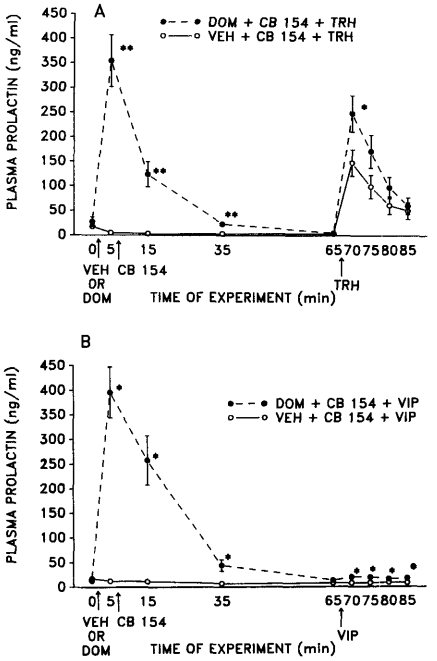

FIG. 1. The effectiveness of TRH and VIP in releasing prolactin following dopamine antagonism in ovariectomized, steroid-treated rats. (A) Plasma levels (mean ± SEM) of prolactin in rats treated with thyrotropin-releasing hormone (TRH, 1.0 μg/rat, iv) following pretreatment with domperidone (DOM, 0.01 mg/rat, iv) and/or 2-Br-α-ergocryptine maleate (CB-154, 0.5 mg/rat, iv). (B) Plasma levels (mean ± SEM) of prolactin in rats treated with vasoactive intestinal peptide (VIP, 25 μg/rat, iv) following pretreatment with DOM and/or CB-154. $*P < 0.05$, $**P < 0.01$; vehicle-injected control vs experimental within each time interval (F test). n = 8–10 animals per group.

effect of VIP on PRL release following domperidone and CB-154 pretreatment was significantly greater than in the control group; however, the injection of VIP resulted in only a 6 ng/ml increase above basal levels of plasma PRL in domperidone-treated animals compared to a 1 ng/ml increase in vehicle-treated control rats.

The administration of sulpiride in steroid-primed OVX rats resulted in a 34-fold increase in plasma PRL 5 min after treatment which was significantly higher than levels observed in vehicle-injected animals (Fig. 2). Plasma PRL declined in sulpiride-injected rats to initial levels in the blood within 65 min of treatment. The administration of TRH stimulated a 20-fold increase in plasma PRL levels in sulpiride-treated rats within 5

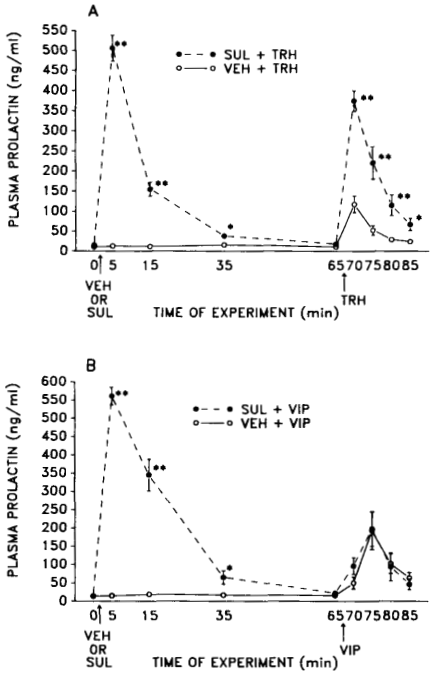

FIG. 2. The effectiveness of TRH and VIP in releasing prolactin following dopamine antagonism in ovariectomized, steroid-treated rats. (A) Plasma levels of prolactin (mean ± SEM) in rats treated with thyrotropin-releasing hormone (TRH, 1.0 μg/rat, iv) following pretreatment with sulpiride (SUL, 0.01 mg/rat, iv). (B) Plasma levels of prolactin (mean ± SEM) in rats treated with vasoactive intestinal peptide (VIP, 25 μg/rat, iv) following pretreatment with SUL. $*P < 0.05$, $**P < 0.01$; vehicle-injected control vs experimental within each time interval (F test). n = 8–9 animals per group.

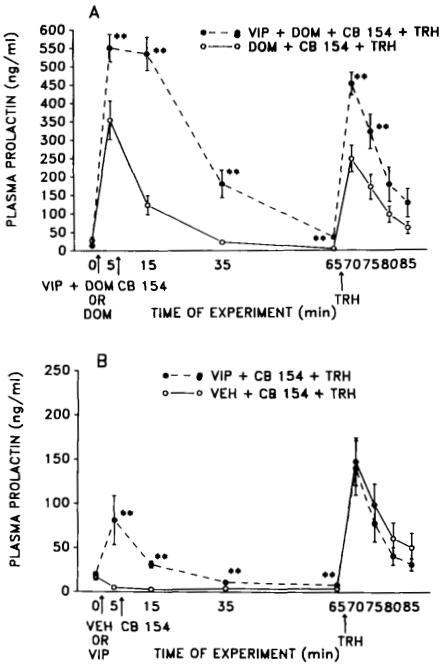

FIG. 3. The effect of dopamine antagonism and/or VIP on the effectiveness of TRH in releasing prolactin in ovariectomized, steroid-treated rats. (A) Plasma prolactin (mean ± SEM) in rats treated with vasoactive intestinal peptide (VIP, 25 μg/rat, iv) and/or domperidone (DOM, 0.01 mg/rat, iv) and 2-Br-α-ergocryptine maleate (CB-154, 0.5 mg/rat, iv) followed by thyrotropin-releasing hormone (TRH, 1.0 μg/rat, iv). (B) Plasma prolactin (mean ± SEM) in rats treated with VIP (25 μg/rat, iv) and/or CB-154 (0.5 mg/rat, iv) followed by TRH (1.0 μg/rat, iv). $**P < 0.01$; vehicle- or DOM-CB-154-injected controls vs experimentals within each time interval (F test). n = 8–9 animals per group.

min compared to a 10-fold increase in vehicle-injected rats (Fig. 2A). The difference between groups remained significant during the 20 min following TRH treatment. The injection of VIP 65 min after sulpiride or vehicle administration (Fig. 2B) resulted in elevated plasma PRL levels which were maximal 10 min after treatment in both groups. However, no significant differences were observed between the control and experimental groups.

The combined effect of dopamine antagonism and VIP on PRL release is shown in Fig. 3A. Five minutes after treatment with domperidone and VIP, plasma PRL levels were significantly higher than those in ani-

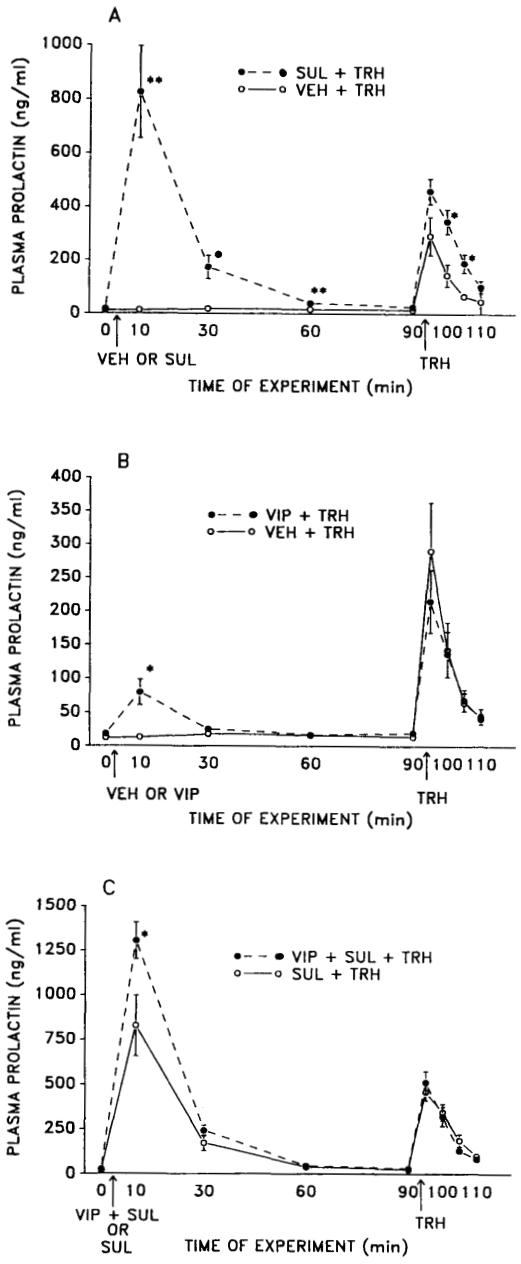

FIG. 4. The effect of dopamine antagonism and/or VIP on the effectiveness of TRH in releasing prolactin in ovariectomized, steroid-treated rats. (A) Plasma prolactin (mean ± SEM) in rats treated with sulpiride (SUL, 0.01 mg/rat, iv) or vehicle (VEH, 0.07% acetic acid in saline) followed by thyrotropin-releasing hormone (TRH, 1.0 μg/rat, iv). (B) Plasma prolactin (mean ± SEM) in rats treated with vasoactive intestinal peptide (VIP, 25 μg/rat, iv) or saline (VEH) followed by thyrotropin-releasing hormone (TRH, 1.0 μg/rat, iv). (C) Plasma prolactin (mean ± SEM) in rats treated with vasoactive intestinal peptide (VIP, 25 μg/rat, iv). $^{*}P < 0.05$, $^{**}P < 0.01$; vehicle- or sulpiride-injected controls vs experimentals within each time interval (F test). $n = 6$–9 animals per group.

mals injected with domperidone alone. Although CB-154 administration lowered PRL levels in the blood in both groups, plasma PRL levels in domperidone–VIP-treated rats remained significantly higher than those in domperidone-injected animals 65 min after experimental treatment. The administration of TRH increased PRL levels in both groups; however, the increase in animals previously treated with domperidone and VIP was significantly greater than in animals treated with domperidone alone at 5 and 10 min after TRH administration. Figure 3B shows the effect on plasma levels of PRL of administering VIP followed by CB-154. The injection of VIP stimulated a fourfold increase in PRL levels within 5 min, which was significantly greater than the levels in rats treated with vehicle alone ($P < 0.025$). Plasma PRL levels induced by VIP treatment decreased after CB-154 treatment; however, PRL levels continued to be significantly higher than the levels in animals injected with vehicle alone 60 min after CB-154 treatment. TRH increased plasma levels in both groups, but no significant differences were observed between the control and experimental groups. This latter experiment was repeated except that sulpiride was used to antagonize dopamine and CB-154 was not given (Fig. 4). Also, TRH was administered 90 min after sulpiride, VIP, or sulpiride + VIP treatments to allow plasma PRL to return to basal levels. Sulpiride significantly increased plasma PRL levels within 10 min (Fig. 4A) and the levels returned to initial values by 90 min after sulpiride. TRH-induced release was enhanced significantly by sulpiride pretreatment. Treatment of a separate group of rats with VIP induced a significant elevation in PRL levels at 10 min followed by a rapid return to pretreatment levels (Fig. 4B). The VIP treatment, however, did not affect TRH-induced PRL release. When VIP and sulpiride were combined (Fig. 4C) the increase in plasma levels of PRL was significantly increased above those in the sulpiride-treated group at

10 min post-treatment; however, the TRH-induced increase in plasma PRL was not significantly different between the two groups.

Discussion. Recently, we demonstrated that the effectiveness of TRH as a PRF in the rat was significantly enhanced by dopaminergic receptor blockade during early pregnancy (16), when PRL surges are also present (21, 22). As part of a study to characterize the role played by the ovarian steroids in this secretory mechanism, we developed the present EB and P treatment protocol (18) to mimic the changes observed in these steroids during early pregnancy (23, 24). This ovariectomized, steroid-treated rat model was used in the current experiments in which we confirmed that dopaminergic antagonism does enhance the effectiveness of TRH. On the other hand, the PRL-releasing effect of VIP was not enhanced by blockade of dopamine receptors. However, VIP augmented the effect of dopaminergic antagonism by domperidone on prolactin release induced by TRH.

Previous observations of the positive influence of DA antagonism on the responsiveness of the lactotroph to TRH were made in lactating rats (11, 14, 15) in an effort to explain suckling-induced PRL release. Grosvenor *et al.* (25–28) demonstrated that a short nursing episode resulted in the "transformation" of AP PRL to a releasable pool from which further release could be more effectively stimulated by one or more hypothalamic PRFs as suckling continued. These same investigators found that transformation could be initiated by the blockade of pituitary DA receptors using domperidone (28, 29). Further, it was shown that pretreatment with DA or the DA agonist CB-154 could inhibit this AP mechanism (25). It has been suggested (11, 14, 15) that the augmentation of the effectiveness of TRH as a PRF following DA blockade may be a secretory manifestation of the lactotroph transformation mechanism.

Using the drug protocol that is shown in Fig. 1, we have previously studied prolactin release under various physiological conditions in the female rat and found that the increase in the effectiveness of TRH after DA blockade was present in all reproductive states where PRL surges are present (i.e., proestrus, early pregnancy, and pseudopregnancy) and absent when PRL surges are not present (diestrus, midpregnancy, and late pseudopregnancy) (unpublished, 16, 17). As a result of these findings we suggested that this AP mechanism may serve as an amplifier of PRL release during surges. On the other hand, the mechanism is also present in lactating rats (25–28) in which surges per se do not occur. However, plasma prolactin is maintained at high levels in lactating rats throughout the day. This prolonged release may also require an amplification system.

In contrast to the potentiated response to TRH following dopamine antagonism, the present study revealed that the iv injection of 25 μg of VIP per animal following domperidone and CB-154 or sulpiride treatment resulted in PRL release qualitatively similar to that of vehicle-injected controls (Figs. 1B and 2B). That the VIP-induced release of PRL was suppressed in domperidone- and CB-154-treated rats but not in sulpiride-treated animals may have been due to the antagonistic effect of CB-154-treated rats but this cannot explain the absence of a potentiated VIP effect in sulpiride-treated rats. It is known that VIP activates adenylate cyclase and increases intracellular cyclic AMP (30–32) whereas dopamine inhibits adenylate cyclase activation (33, 34). Indeed, a direct and dose-related inhibitory action by DA on VIP-induced increases in AP cAMP and PRL release has been demonstrated in prolactinomas *in vitro* (32). Because of the apparent antagonism between the mechanisms of action of VIP and DA at the lactotroph, one or both of the following events would be required if VIP is to be viewed as a potent PRF under physiological conditions when dopamine is critical to the overall control of PRL release: (1) the decrease in DA levels at the AP during periods of increased PRL release must be dramatic and sustained, or (2) hypophysial portal blood levels of VIP must increase sufficiently to override DA inhibition. Various studies have demonstrated that the decrease in DA activity at the AP during suckling or mammary nerve stimulation is transient in nature (35–37) and over the course of nursing episode may even be elevated at some sampling periods. Further, some investigators have found that during the PRL surges on proestrus (38) and during pseudopregnancy (39) there may not be a sig-

nificant decrease in dopamine arriving at the AP or, if a decrease does occur, it is not dramatic. Whether sufficient VIP is released into the portal blood to override the effects of dopamine under physiological conditions is not clear. In the current studies 25 μg VIP/rat induced prolactin release (Figs. 2B, 3B, and 4B); however, other studies in our laboratory have shown that VIP at 1 and 10 μg per animal, iv, without CB-154 pretreatment were completely ineffective in stimulating PRL release in lactating or E-treated OVX rats. In earlier studies we observed that VIP at 1.0 μg/rat, iv, decreased blood pressure 25% within 10 sec (unpublished observations) indicating the preparation was biologically active at a dose that released no prolactin. Although VIP levels in hypophysial portal blood have been shown to be higher than in peripheral blood (2–4) implying release from the median eminence, attempts at demonstrating elevations in VIP release into the hypophysial portal blood following hypothalamic electrical stimulation (2), L-5-hydroxytryptophan (5-HTP) treatment (4), or during the estrous cycle of the rat (2) have provided conflicting results.

Although these arguments seem to favor a limited role of VIP as a PRF under the conditions we have employed, we attempted to test the hypothesis by another pharmacological manipulation. To produce a short period during which dopamine tone to the AP is decreased using the antagonist domperidone requires administration of a dopamine agonist such as CB-154 since domperidone is a long acting D_2 receptor blocker that elevates plasma PRL levels for at least 2 hr (40). Such a requirement is circumvented with the use of a shorter acting DA receptor antagonist such as sulpiride. We examined whether sulpiride could enhance the prolactin-releasing activities of TRH and VIP (Figs. 2 and 4). The results demonstrated that, although both hypothalamic peptides stimulated PRL release, sulpiride pretreatment enhanced only the response to TRH.

Although TRH is considered by many to be one of the leading candidates for a physiological PRF, the results of various studies have been conflicting and controversial (10–13, 41–43). Further, some investigators have found significant PRF activity in hypothalamic extracts that do not contain TRH (28, 44, 45). TRH does, however, have two important characteristics which we have not been able to demonstrate for VIP (present study) or oxytocin (unpublished observations), another putative PRF (46). First, the PRF action of TRH appears to be minimally affected by DA or DA receptor agonists (14–18, present study). This is probably because TRH stimulates PRL release by utilizing inositol trisphosphate to mobilize intracellular calcium (47, 48) and not cAMP. Second, the prolactin-releasing effect of TRH is augmented after DA suppression or withdrawal.

If TRH is the prolactin-releasing factor responsible for sustained prolactin release (i.e., following suckling and during surges) when dopamine tone at the pituitary is high, then what is the possible role of VIP? One possibility is that VIP is a releasing factor under conditions when dopamine tone is not significant. It may also be significant in combination with other releasing factors. Another possibility is that VIP acts in concert with a brief decrease in dopamine to sensitize the lactotroph to TRH. This possible effect of VIP on TRH-induced PRL release was examined (Figs. 3 and 4). Our data show that VIP treatment alone did release PRL but did not increase the effectiveness of TRH (Figs. 3B and 4B). However, the combination of VIP and dopamine antagonism produced by domperidone increased significantly the effectiveness of TRH to release PRL compared to the TRH response following domperidone alone (Fig. 3A). This suggests that VIP may be a part of the battery of hypothalamic signals that transforms prolactin from a prerelease pool to a releasable pool within the pituitary. That a similar effect was not seen when dopamine was antagonized with sulpiride (Fig. 4C) may have been due to the timing of the TRH administration (90 min after sulpiride vs 60 min after domperidone) or the amount of prolactin released by sulpiride and VIP (peak level of 1250 ng/ml) compared to the domperidone–CB-154–VIP combination (peak level of 550 ng/ml). It is possible that the "transformed" pool was depleted by sulpiride-induced release but not by the smaller domperidone-induced release.

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