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ROLE OF MONOKINES IN CONTROL OF ANTERIOR PITUITARY HORMONE RELEASE

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Introduction

It has been known as far back as 1936 from the pioneering work of Selye (1) that noxious stimuli of one type or another, activate the release of ACTH which in turn releases adrenal cortical steroids. These then bring about a series of reactions in the body which consist of thymic involution, decrease in size of lymph nodes, lymphopenia, and eosinopenia (1). The role of the nervous system in these phenomena was not established at that time; however, in the early '50s it became apparent that hypothalamic lesions, particularly in the median eminence of the tuber cinereum would abolish the release of ACTH and adrenal steroids resulting from stress (2). Conversely, stimulation of the hypothalamus could evoke release of ACTH followed by release of adrenal steroids (3).

Early studies with adrenal steroids and ACTH indicated that these steroids had predominantly an immunosuppressive effect. This has been amply confirmed by later work now that the immune system is much better understood (4). Although hypercorticalism leads to suppression of immune responses, levels of the steroids which would be found under resting conditions may actually promote certain immune responses (5). The response to stress extends beyond the activation of ACTH secretion to the activation of prolactin (PRL) and growth hormone (GH) release in man (6). PRL and GH release is augmented by nearly all stresses in lower forms; however, the rat is an exception in that stress, instead of stimulating, inhibits GH release (7).

The hypophysectomized animal in general shows deficient immune responses which can be corrected by the administration of either GH (8) or PRL (9). Since these peptides are related chemically, it may be that the receptors which mediate these responses respond to portions of these two peptides that are similar in three dimensional structure. Thus, ACTH and adrenocortical hormones are immunosuppressive, whereas GH and PRL augment immune responses.

The hypothalamus may also modulate immune responses by the stimulation of the sympathoadrenal system. Beta receptors have been found on most immune responsive cells and in general appear to have a suppressive action on immune responses (10). Since stress activates the sympathoadrenal system, this system would appear to participate with

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and augment the immunosuppression induced by the pituitary-adrenocortical system.

With the discovery that ACTH is produced as a proopiomelanocortin molecule, which is processed differentially in different tissues, it appeared of interest to see whether β-endorphin was also released by the pituitary as well as ACTH. This has turned out to be correct (11), and β-endorphin appears to have the capability to modify immune responses (12). If this can be shown to be true at levels of the peptide which are found in the circulation in stress, then this may be another pathway for neuroimmunomodulation. Alternatively, recent evidence suggests that ACTH and endorphins (13) can actually be produced in lymphocytes where they may modulate these responses.

Following infection, the introduction of bacterial endotoxins, or most immunization procedures, a stress-like response of the hypothalamic-pituitary unit occurs (14). Therefore, in the acute phase one would have the stimulation of ACTH and adrenal corticoids tending to suppress the immune response, which would be counterbalanced by the stimulatory effects of GH (except in the rat) and PRL released during stress. Although there is abundant evidence for adrenal steroid receptors on immune cells (15), this has not been shown clearly for PRL and GH, and their effects may be mediated particularly by actions on the thymic cells (4). Multiple pathways mediate the activation of the hypothalamic-pituitary unit that takes place during stress (16). This can occur via emotional stimuli, painful stimuli, damage to tissue that produces pain and products of the immune cells themselves may evoke the response.

For example, exogenous pyrogens such as typhoid vaccine evoke the release of endogenous pyrogens that circulate through the blood to the hypothalamus to evoke fever by affecting the temperature regulating centers. Chowers *et al.* (17) demonstrated in dogs that injection of bacterial pyrogen evoked fever after a delay. The fever was presumably induced by endogenous pyrogen and was correlated in time with an elevation of plasma cortisol indicative of an activation of the pituitary-adrenal system by endogenous pyrogen.

Interleukin-1

The principal endogenous pyrogen is now known to be interleukin-1 (IL-1), a peptide having a molecular weight of about 17,000. It has become available for study, and it is apparent that peripheral administration of the peptide activates ACTH secretion (18,19). Intravenous administration of IL-1 increases plasma ACTH in the rat presumably by evoking release of corticotrophin releasing factor (CRF). This supposition is supported by recent experiments which showed that following intravenous administration of IL-1, there was an increase in CRF in portal blood and a borderline increase in vasopressin as well (19). Both of these peptides are capable of directly stimulating a release of ACTH from the pituitary gland, and vasopressin potentiates the action of CRF to release ACTH (20). In other studies, antisera directed against CRF have been shown to block the response to systemic administration of IL-1, again indicating that the response may be mediated by release of CRF (18,19).

A possible action of IL-1 to affect ACTH release directly is controversial. In AT-10 tumor cells, which consist of corticotrophs, IL-1 stimulates ACTH release *in vitro* (21). IL-1 has been found in three studies to have no effect on release of ACTH from normal pituitary cells *in vitro* (18,19,22), and furthermore it failed to alter the response to CRF (18). However, in one study a dose-related release of ACTH was found in monolayer cultured pituitary cells (23). The reason of these discrepant results is not apparent.

We have studied the effects of IL-1 on the release of other anterior pituitary hormones. Natural IL-1 was injected into the third ventricle of conscious male rats. Control animals were injected with the solvent vehicle [0.9% NaCl (saline)]. There was

Table 1
Effect of Intraventricular Injection of Saline and of IL-1 at Doses of 5 and 25 ng on Rectal Temperature (°C) in Male Rats*

	Time (min)		
Treatment	0	120	240
Saline	37.1 ± 0.4	34.9 ± 0.05†	34.6 ± 0.8‡
IL-1, 5 ng	36.8 ± 0.3	36.3 ± 0.9	35.8 ± 1.1
IL-1, 25 ng	37.3 ± 0.1	38.4 ± 0.2†	38.0 ± 0.3

*Time zero was approximately 30 min prior to iv injection. All values are mean ± SE (8 rats/group).
†$P < 0.005$ *vs.* time 0 value.
‡$P < 0.02$ *vs.* time 0 value.

a decline in body temperature in the control rats at 120 and 240 minutes after initiation of blood sampling (Table 1). Following the lowest dose of IL-1 [5 ng (0.3 pmol) in 2.5 μl], rectal temperature was significantly higher than in the saline-injected group at 120 minutes but was no longer significantly elevated at 240 minutes. Thus, this dose of IL-1 produced a borderline elevation of body temperature. The higher dose of 25 ng (1.5 pmol in 2.5 μl) of IL-1 evoked a highly significant elevation in body temperature at 120 minutes following initiation of blood sampling ($P < 0.005$). Temperature remained elevated at 240 minutes. Thus, the higher dose produced a frank fever.

After injection of saline into the third ventricle, plasma GH levels declined significantly at 15 minutes and remained low for the duration of the experiment; whereas following intraventricular injection of 5 ng of IL-1, plasma GH increased at 15 minutes and remained elevated for the duration of the experiment (Figure 1). These values were significantly above those in the saline-injected controls, and the area under the GH release curve was significantly elevated above that in the saline-injected group. Surprisingly, this effect was not evident following the injection of 25 ng of IL-1, and in this instance the results were almost superimposable on those of the animals that received intraventricular saline.

Plasma thyrotropin (TSH) levels did not significantly change after intraventricular injection of saline; however, after injection of 5 ng of IL-1, there was a decline in plasma TSH beginning at 15 minutes that achieved statistical significance by 30 and 60 minutes whether compared with plasma TSH in the saline-injected animals or the starting values. Similarly, the area under the plasma TSH curve was significantly less in the rats that received this dose of IL-1 than in the saline-treated rats. Although TSH values were lower following 25 ng of IL-1, these changes were no longer significant on comparison with saline-injected control values. They were also not significantly different from the values obtained with the lower dose of IL-1. The results with this higher dose were not significant when the area under the release curve was calculated.

Intraventricular injection of saline was followed by a slight but not significant decline in plasma PRL at 15 minutes. Values tended to return toward baseline by 120

minutes. After the lower dose of IL-1 (5 ng) was injected intraventricularly, an elevation of plasma PRL was observed at 60 minutes and thereafter. However, the variance was large, so these values were not significantly different from starting values or those of the saline-injected groups. This tendency to elevation was not observed following IL-1 at the dose of 25 ng. Similarly, when the area under the plasma PRL release curve was calculated, although the values were higher in the animals injected with 5 ng of IL-1, this change was not found to be significant and there was no alteration in the animals receiving the higher dose compared to the saline-injected controls.

When we compared the values at various times following injection of saline in order to calculate the maximal post-treatment increase in plasma PRL values for each animal, we found that the mean maximum increase in the saline-injected animals was 3.2 ± 2.0 ng/ml (mean ± SE), indicating no significant increase in PRL at any time following the injection of saline. However, in the animals injected with 5 ng of IL-1, the maximum mean increase in plasma PRL following the injection was 10.9 ± 3.5 ng/ml, a significant increase ($P = 0.02$). On the other hand, the maximum increase following the higher dose of IL-1 of 4.8 ± 2.5 ng/ml was not statistically significant (24).

We expected to find a stress-like pattern of hormonal release following the intraventricular injection of IL-1. Instead, we found a different picture. In general, stress decreases GH release in the rat (7); yet, we found that although there was an apparent slight stress effect of intraventricular injection of the saline diluent, as reflected in significant declines in plasma GH, the effect of IL-1 at the 5 ng (0.3 pmol) dose was to elevate plasma GH significantly above the values in the saline-injected controls. This dose, although not producing a febrile effect, prevented the decline in body temperature that was seen in the control animals following intraventricular injection of saline and multiple blood sampling. We do not know the cause of this small decline in temperature, but it may be related to the blood sampling and the maintenance of the animals in single

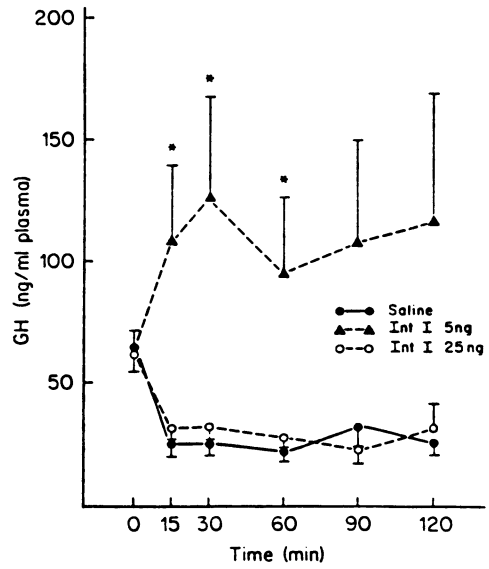

Figure 1. The effect of injection of the saline diluent or different doses of interleukin 1 (Int 1) in 2 μl of saline (0.9% NaCl) on plasma growth hormone in conscious male rats. Values in this and subsequent figures are mean ± 1 SE. *$P < 0.05$ *vs.* saline-injected controls.

cages so that heat loss was increased. The failure of temperature to decline in the interleukin-injected animals indicates that we had reached a dose that was biologically effective in raising body temperature.

It was very surprising that when the dose of IL-1 was increased to 25 ng (1.5 pmol), injected intraventricularly, which was associated with a clear febrile response, the response of GH was muted and no longer significantly different from values in the saline-injected animals. We have no explanation for this effect; however, it would appear that when frank elevation of temperature occurs, the response appears to be reversed.

The mechanism of the interleukin-induced elevation in GH is not known but may reflect either a decrease in release of somatostatin or an increase in the release of GH-releasing factor, or both changes may be operative. In previous studies of the effect of IL-1 on pituitary cells incubated *in vitro*, no effect on the release of GH was demonstrable in one case (22) whereas in the other experiment, a dose-related stimulation of IL-1 on GH release was obtained. Consequently, in our studies in which the hormone was injected intraventricularly, it is likely that the effects were mediated within the hypothalamus; however, we cannot rule out the possibility that sufficient amounts of the protein reached the anterior pituitary to stimulate GH release directly. Arguing against this possibility is the fact that the stimulation of GH vanished at higher doses which should have had a greater effect at the pituitary level. Therefore, it is quite probable that the effects observed were indeed due to hypothalamic action of IL-1.

Stress, in general, results in an inhibition of TSH release (7), so we expected to see a decrease in TSH in the interleukin-injected animals; and indeed this was found with significant effects at the 5 ng dose. On the other hand, there was no change in plasma TSH in the animals receiving saline. Again, as in the case of GH, there was no effect from the higher dose (25 ng) of IL-1. We speculate that frank pyrexia somehow interferes with the effects of the peptide. Presumably, the effect observed with the 5 ng dose is due to decreased TRH release from the hypothalamus.

We were expecting an increase in PRL following administration of IL-1, since this is a usual pattern with stress (7). However, there was great variability in the response, and significance was achieved only if one considered the maximal increment in PRL following injection of the 5 ng dose. Again, the response to the 25 ng dose was less and did not quite achieve statistical significance. Our results extend the pattern of hormonal response to IL-1 to include a stimulation of GH and PRL release and an inhibition of TSH release. They also show that as frank pyrexia develops, the hormonal responses are muted.

It will be necessary to do further experimentation with incubation of hemipituitaries and dispersed pituitary cells *in vitro* to distinguish clearly between the probable hypothalamic effect that we have seen and possible direct effects if IL-1 on the anterior pituitary.

Tumor Necrosis Factor (Cachectin)

An exciting recent development has been the discovery of the macrophage hormone, cachectin, which has recently been shown to be identical to tumor necrosis factor (25). It has a molecular weight of 17,000 daltons which is similar to that IL-1. Cachectin is released in response to infection, and the most effective promoter of its release is bacterial endotoxin, which is a lipopolysaccharide. Exposure of macrophages *in vitro* to endotoxin results in the synthesis of mRNA that initiates the synthesis of cachectin. This is released from the macrophages and has many effects in the organism. Low concentrations can activate phagocytosis and may play a beneficial role in combating infection; however, overwhelming infection results in massive stimulation by endotoxin and a discharge of large quantities of the hormone into the circulation where it has many

adverse effects (27). It acts on endothelial cells to release IL-1 (27). IL-1 as well as cachectin act on the temperature regulating centers to induce fever. There is increased coagulability of the blood which can result in thrombosis. High concentrations alter endothelial permeability with resultant extravasation of blood. Permeability of cell membranes is altered with resultant changes in muscle membrane potential and uptake of water by cells. There is a blockade of lipoprotein lipase resulting in a failure to clear triglycerides and hypertriglyceridemia. Many other metabolic changes occur that represent manifestations of the so-called toxic shock syndrome produced by bacterial lipopolysaccharides. In fact evidence is accruing, based on passive immunization against cachectin, that the toxic shock syndrome is caused by release of massive quantities of this hormone into the circulation (26).

In dogs, hormonal changes have been demonstrated as evidenced by increases in circulating epinephrine, norepinephrine, cortisol, and glucagon following injection of cachectin (26). Therefore, it is apparent that cachectin can stimulate ACTH secretion; however, there have been no studies to determine the mechanism by which this effect takes place. It could be a result of afferent stimuli impinging on the hypothalamus from the multitudinous peripheral effects of the hormone, or a result of effects on the temperature regulating centers that in turn cause release of CRF and/or vasopressin as appears to be the case for IL-1. There may also be a direct effect on the pituitary.

We have carried out an extensive study of the effects of cachectin on hypothalamic-pituitary function in the rat. As in the case of IL-1, rectal temperature rose at the first measurement 1 hour after the intraventricular injection of tumor necrosis factor (TNF). There was no dose-response relationship since the responses to the 5 ng (0.3 pmol) dose and 100 ng (6.0 pmol) dose were similar.

In this case there was no significant decline of plasma GH following intraventricular injection of the saline diluent, but there was a significant increase in plasma GH in animals injected with the highest dose of 100 ng of TNF, which was significant only at 1 hour after injection.

Plasma PRL was not significantly altered by intraventricular injection of saline; however, following intraventricular injection of TNF at either the lower 5 ng or higher 100 ng dose, plasma PRL was elevated slightly, and the maximal increases from basal values were significant for both doses with a greater significance ($P < 0.025$) for the 100 ng dose.

There was no significant alteration of plasma TSH in control animals; however, TSH became significantly lower by 3 hours following both doses of TNF. This decrease persisted until 4 hours in the case of the lower dose, but TSH levels continued to decline and remained significantly lower at 6 hours following the higher dose of the peptide.

Plasma ACTH was slightly but significantly elevated by the higher dose of TNF but was unaltered by intraventricular injection of the diluent.

To determine the possible role of prostaglandins in these responses, the animals received a microinjection of indomethacin into the third ventricle, at a dose which had previously been found to block the release of LH in ovariectomized rats. Indomethacin had a significant suppressive effect on the response of PRL and TSH to cachectin but failed to alter significantly the response of ACTH to the peptide.

To determine whether or not the effects found with intraventricular injections were mediated by alterations in the secretion of releasing factors or by a direct action on the pituitary itself, the effects of several concentrations of TNF were tested for their ability to alter anterior pituitary hormone release from overnight cultures of dispersed anterior pituitary cells or of hemipituitaries in static incubations.

Preliminary experiments were carried out with concentrations of cachectin of 10^{-10} and 10^{-9} M to determine the optimal time for measurement of hormone release from dispersed pituitary cells of male rats. Cachectin failed to alter either TSH or GH release

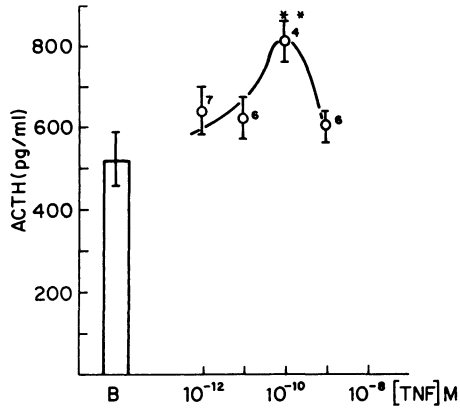

Figure 2. Effect of various doses of cachectin (TNF) on the release of ACTH from hemipituitaries incubated *in vitro* for 2 hrs. B represents the basal release. Numbers beside the mean indicate the number of flasks incubated. $^{**}P < 0.025$ *vs.* basal release. Reproduced from Milenkovic *et al.* (28) with permission of the authors.

at 30 or 60 minutes but significantly increased the release of both hormones at both doses following 120 minutes of incubation. Consequently, this latter period was chosen for further study.

Cachectin significantly stimulated release of ACTH with a bell-shaped dose-response curve; the maximal significant response occurred at 10^{-10} M (Figure 2). This contrasted with a failure of the peptide to stimulate consistently prolactin release from the same hemipituitaries. There was stimulation in two of four experiments. The peptide also significantly stimulated GH release from these same hemipituitaries with a bell-shaped dose-response curve as in the case of ACTH (Figure 3). In this case the maximal effect occurred at a very low concentration of 10^{-12} M. Similarly, there was a dose-related release of TSH except that the peak was sharper than in the case of GH,

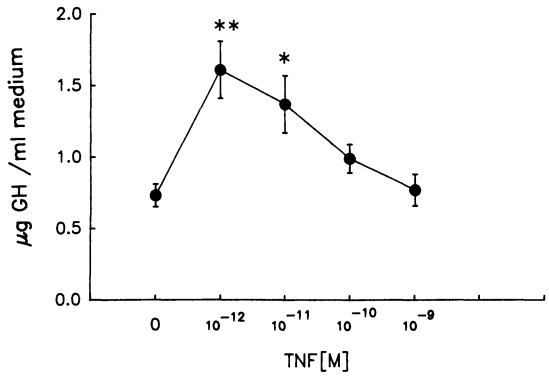

Figure 3. The effect of cachectin (TNF) on GH secretion from hemipituitaries incubated *in vitro*. $^{**}P < 0.01$ *vs.* control; $^{*}P > 0.05$ *vs.* control.

and no stimulation occurred except at the low dose of 10^{-12} M (Figure 4).

In the case of dispersed anterior pituitary cells, cachectin induced a dose-related and significant stimulation of ACTH release with the maximal effect occurring at a higher concentration than with hemipituitaries of 10^{-9} M. As with hemipituitaries there was inconstant stimulation of PRL release from the dispersed cells. TSH release was stimulated in a dose-related fashion, with highly significant stimulation at both 10^{-10} and 10^{-9} M. The stimulations with these doses were not significantly different from each other. Similarly, stimulation of GH release occurred, which reached statistical significance at 10^{-10} M; however, in this case there was a tendency for a bell-shaped curve and stimulation with 10^{-9} M was not significant. It is noteworthy that the concentration required to stimulate the release of each of these three pituitary hormones in the dispersed cell system was greater than that needed with hemipituitaries.

In an attempt to determine the mechanism of these effects, the responses were determined in the presence or absence of somatostatin (10 nM) (Figure 5). Somatostatin suppressed basal GH release and blocked the stimulatory effect of 10^{-10} cachectin on GH release. In the case of PRL there was no significant stimulatory effect of this dose of cachectin on prolactin release. In the presence of somatostatin basal prolactin release was not significantly reduced, and a highly significant stimulatory effect occurred with the addition of cachectin. In the case of TSH, somatostatin failed to alter the basal or stimulated release by cachectin.

To evaluate the role of cyclic AMP in the mechanism of cachectin stimulation, cachectin was incubated with the cells, and cyclic AMP in the cells was measured at the end of the experiment along with the various hormones. Cachectin lowered cell cyclic AMP concentrations in a dose-related fashion with the first significant lowering occurring at 10^{-9} M TNF (Figure 6). In the presence of somatostatin, cyclic AMP levels were lowered but now instead of lowering them further, TNF elevated the levels significantly (Figure 5). This was associated with the changes in hormone release mentioned before, *viz.*, that in the presence of somatostatin the GH response to cachectin was blocked but that of TSH was unaltered, but in the presence of somatostatin the release of PRL was dramatically increased from a nonsignificant increase to a highly significant one.

Lastly, to evaluate the role of prostaglandins in the *in vitro* effect of cachectin on pituitary hormone release, indomethacin was present at a concentration of 10 μM during both preincubation and incubation periods. Indomethacin did not alter basal release of the various hormones but completely blocked the stimulatory effect of 10^{-10} M cachectin

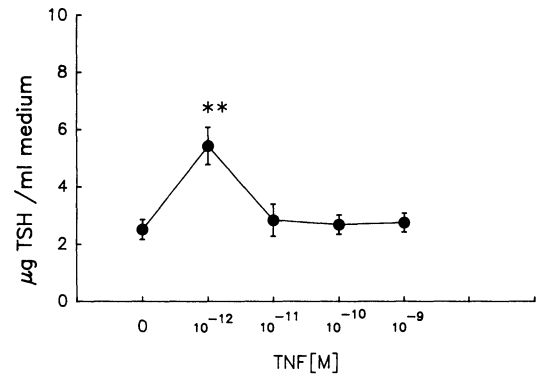

Figure 4. The effect of cachectin (TNF) on TSH secretion from hemipituitaries incubated *in vitro*. $^{**}P < 0.01$ *vs.* control.

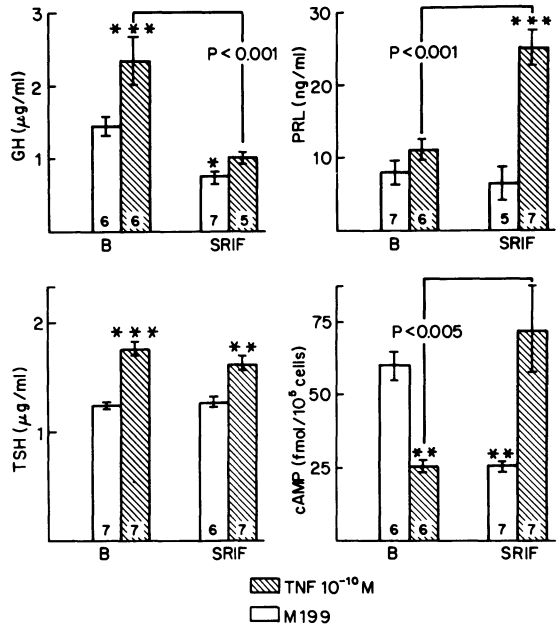

Figure 5. Effect of cachectin (TNF) (10^{-10} M) on the release of hormones from dispersed pituitary cells in the presence or absence of somatostatin (SRIF) (10 nM). Medium 199 (M199) served as the control. The effects of these treatments on the cyclic AMP content of the cells at the end of the incubation is also shown (*lower right panel*). $*P < 0.05$ *vs.* control; $**P < 0.025$ *vs.* control; $***P < 0.005$ *vs.* control. Reproduced from Milenkovic *et al.* (28) with permission of the authors.

on GH and TSH release, but did not significantly decrease the ACTH-releasing action of the peptide (Figure 7) (28).

After the intraventricular injection of cachectin, results were similar to those obtained with IL-1 (0.3 pmol) with pyrexia. There was no significant further elevation of body temperature following the higher dose.

The effects of cachectin on pituitary hormone release following its intraventricular injection were similar but slightly less pronounced than those obtained with IL-1. We obtained only a slight increase in GH release following the higher dose (6 pmol) and have not yet had a satisfactory experiment with the lower dose. The elevation of PRL was modest. The most striking effects were obtained in the case of TSH, which was significantly lowered by both doses with a prolonged lowering obtained with the higher dose (6 pmol). There was also a significant stimulatory effect on ACTH release at the higher dose. The effects on GH, PRL, TSH, which followed intraventricular injection of cachectin, may have been due in part to the release of prostaglandins since the cyclooxygenase inhibitor, indomethacin, which should inhibit prostaglandin synthesis, attenuated the effects except in the case of ACTH. In view of the relatively long period before the induction of any of these responses *in vivo*, they could be mediated either by cachectin itself or by IL-1 released from astrocytes (25).

In the case of GH, ACTH, and PRL the actions of the peptide on the release of pituitary hormones *in vitro* had the same sign as those occurring following intraventricular injection. Thus, it is possible that the *in vivo* effects may have been due

to actions on the pituitary itself after uptake of the peptide from the third ventricle, diffusion into the primary capillary plexus of the portal vessels, and delivery to the gland. However, in view of the long latency of these effects and the much lower concentrations that would reach the pituitary than those which would reach the adjacent hypothalamic tissue, we are inclined to believe that these actions following intraventricular injection are mediated at the hypothalamic level, perhaps in part by prostaglandins, which may also induce the pituitary responses. In the case of TSH in which intraventricular injection lowered plasma TSH while *in vitro* incubation with pituitaries elevated it, the hypothalamic action obviously could not be related to any effect on the pituitary *in vitro*.

It is interesting to note that in the case of the actions on pituitary cells that wherever a comparison was made, the hemipituitaries were more sensitive to TNF stimulation than were the dispersed anterior pituitary cells. The reason for this enhanced sensitivity of hemipituitaries is not known but may be related to a paracrine interaction between the various cell types in the hemipituitaries, whereas in the case of the dispersed cells which are isolated from each other, the sensitization by paracrine interaction was not present. Alternatively, the tissue dispersion with trypsin may have damaged cell membrane receptors which would also result in diminished sensitivity.

In initial attempts to determine the mechanism of the stimulatory actions of cachectin, cyclic AMP was determined in the cells at the end of the incubation and a dose-related lowering of cyclic AMP concentrations was found. Reversal of this lowering action of cachectin by somatostatin was also accompanied by stimulation of release of PRL. Somatostatin itself lowered cyclic AMP, as might be expected from prior results. It acts on a variety of cells in the pituitary gland to lower cyclic AMP, and the lowering is accompanied by decreased release not only of GH but also, at least at high doses, of PRL and TSH (29). The stimulatory action of cachectin on PRL release in the presence of somatostatin may be brought about by an elevation of cyclic AMP, which may have occurred in the lactotrophs, but only in the presence of somatostatin plus cachectin. Therefore, elevation of cyclic AMP may be a factor in promoting PRL release in response to cachectin. Indeed, exogenous cyclic AMP can stimulate PRL release.

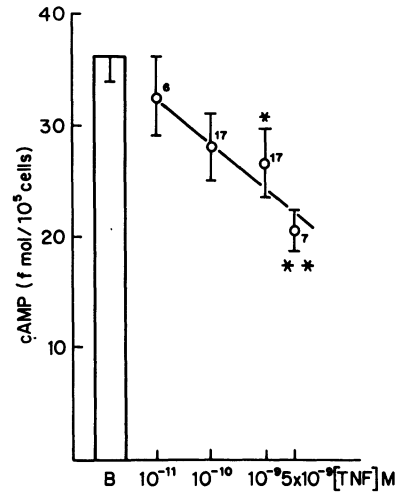

Figure 6. Pituitary cell cyclic AMP concentrations after incubation with various doses of cachectin (TNF). $*P < 0.05$ *vs.* basal; $**P < 0.025$ *vs.* basal. Reproduced from Milenkovic *et al.* (28) with permission of the authors.

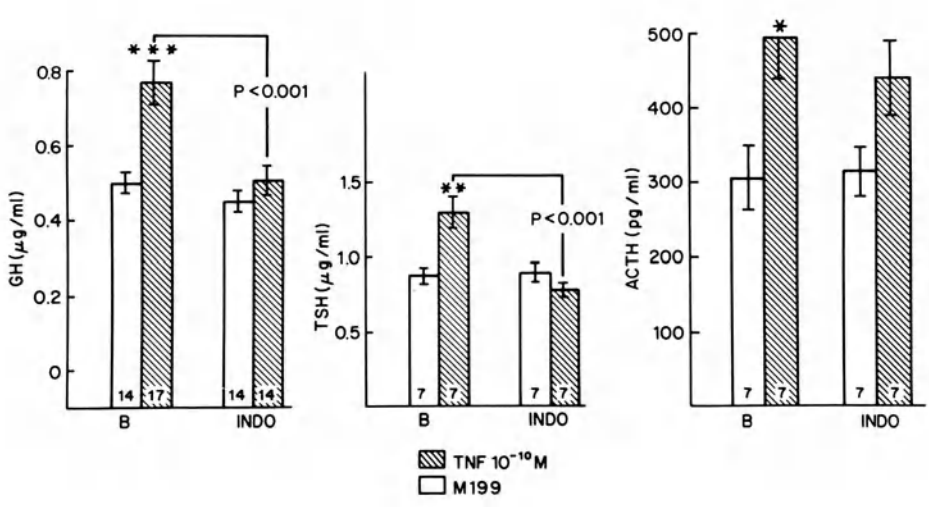

Figure 7. Effect of indomethacin on the release of pituitary hormones by cachectin (TNF) (10^{-10} M). Indomethacin (INDO) was present in both preincubation and incubation periods at 10 μM. *$P < 0.05$ *vs.* basal; **$P < 0.025$ *vs.* basal; ***$P < 0.005$ *vs.* basal. Reproduced from Milenkovic *et al.* (28) with permission of the authors.

Although indomethacin did not alter basal hormone release, it blocked the releasing action of cachectin on both GH and TSH release *in vitro*. These results suggest that cachectin interaction with receptors on pituitary cells leads to activation of prostaglandin synthetase, which generates the release of prostaglandins that in turn stimulate release of GH and TSH. Earlier experiments have shown that prostaglandins can indeed release these various pituitary hormones by direct action *in vitro*. Thus, the results support the hypothesis that the releasing action of cachectin is mediated at least in part by prostaglandins. Recently, experiments have been reported which indicate that cachectin can stimulate endogenous production of prostaglandin E_2 by macrophages and that this action is blocked by the cyclooxygenase inhibitor indomethacin, which suppresses the metabolic activation of macrophages. It appears that the action of cachectin on pituitary cells may be similar to that on macrophages. The failure of indomethacin to block the ACTH-releasing action of cachectin is puzzling and suggests a role for other intracellular mediators in this action. The proposed interactions at the hypothalamic and pituitary level of IL-1 and TNF are illustrated in Figure 8.

Gamma Interferon

The number of monokines whose structure has been determined and synthetic versions made has increased by leaps and bounds in the last several years. Another available, important monokine is gamma interferon, and we have begun to study the possible effects of this monokine on hypothalamic-pituitary function as well.

Injection of human recombinant gamma interferon into the third ventricle of conscious male rats at a dose of 5 ng lowered plasma GH levels by 15 minutes. They were significantly lowered by 30 minutes and remained low for the 2 hour duration of the experiment on comparison with plasma GH levels in the saline-injected controls.

Interestingly, the higher dose of 25 ng of gamma interferon did not produce any significant alteration on comparison with saline-injected controls. The maximal decline in plasma GH was significantly increased at the lower but not at the higher dose compared to the decline in saline-injected animals. Similarly, plasma TSH was decreased by 60 minutes and significantly lowered at 90 and 120 minutes following intraventricular injection of the lower dose of 5 ng of gamma interferon, but the values in animals receiving 25 ng were almost superimposable on those of the saline-injected control groups. Neither dose of gamma interferon altered plasma PRL levels. Plasma ACTH was elevated only by the higher dose of interferon. There was slight hyperthermia, present only at 4 hours following intraventricular injection of both doses.

To determine whether or not these were hypothalamic or pituitary actions of gamma interferon, the peptide was incubated with hemipituitaries incubated *in vitro*. It had little effect on the release of pituitary hormones, but at a relatively high dose (10^{-8} M) stimulated ACTH release. There was no other significant effect on pituitary hormone release *in vitro*.

Thus, it appears that gamma interferon alters hypothalamic function to produce changes in pituitary hormone release. The results are unique for this monokine and different from those obtained with either IL-1 or cachectin. The principal action of gamma interferon appears to be on structures near the third ventricle to alter pituitary hormone release (30).

It will be important to examine the actions of other monokines as they become available. The picture is much more complicated than earlier envisioned, and it is probable that the response of the hypothalamic-pituitary axis to infection is brought about

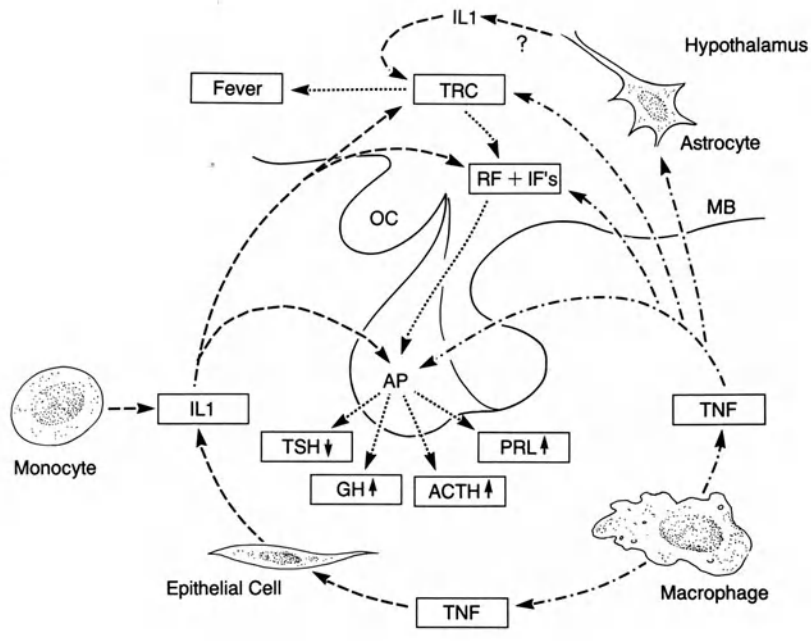

Figure 8. Summary of the proposed interactions of IL-1 and cachetin (TNF) on the hypothalamus and anterior pituitary gland. Abbreviations: TRC: temperature regulating centers; RF: releasing factor; IF: inhibiting factors; MB: mammallary bodies; OC: optic chiasm; AP: anterior pituitary. Reproduced from McCann *et al.* (31) with permission of Alan R. Liss, Inc.

by complex interactions of monokines acting both on the hypothalamus and pituitary directly.

Summary

It has long been known that endogenous pyrogen, released as a result of injection of typhoid vaccine or in response to infection, produces fever and increases ACTH secretion. Recent studies have indicated that endogenous pyrogen is, at least in part, IL-1. This monokine has now been shown to activate the release of ACTH by a hypothalamic mechanism with release of CRF and possibly vasopressin, which stimulates the corticotrophs. There may also be a pituitary action to stimulate the release of ACTH directly. In our experiments we showed that IL-1 at low but not higher doses appears to act intrahypothalamically to stimulate GH and PRL release and to inhibit TSH release.

In the meantime, another monokine, cachectin, was isolated and its structure determined. We have found that this monokine can act following its third ventricular injection to stimulate ACTH, PRL, and GH release and to inhibit TSH release, at least in part, by release of prostaglandins since indomethacin, an inhibitor of prostaglandin synthesis, produced a blockade of the responses except for those of ACTH. This peptide also has highly potent effects to alter pituitary hormone release by direct action on the pituitary to stimulate ACTH, GH, and TSH and to a slight extent PRL release. These actions appear to involve prostaglandins since indomethacin blocks all of the effects except for the effect on ACTH secretion. This monokine also produces a dose-related lowering of anterior pituitary cyclic AMP levels. When the monokine was incubated along with somatostatin, the lowering of cyclic AMP was reversed, and a potent PRL-releasing effect of the monokine was visible.

We have begun studies with a third monokine, gamma interferon, which indicate that it stimulates ACTH release but suppresses plasma GH and TSH levels by a hypothalamic action.

It is apparent that these various monokines have powerful effects to alter hypothalamic-pituitary function and that they probably mediate most of the effects of infections on the release of anterior pituitary hormones.

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