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Pharmacological Studies of the Involvement of Hypothalamic Prostaglandins in the Regulation of Thyrotropin Secretion

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A case is made for the involvement of pituitary prostaglandins (PGs) in the regulation of thyrotropin (TSH) secretion by citing recent evidence that TSH release *in vivo* and *in vitro* is enhanced by treatment with exogenous PGs and is inhibited by drugs (e.g., indomethacin) that block PG synthesis. Pharmacological studies were then performed to test the hypothesis that hypothalamic PGs also affect TSH secretion indirectly via the appropriate hypothalamic hormones that regulate pituitary secretion. The inhibition of thyroidectomy-induced TSH secretion was used as an endpoint in choosing the best of several drugs purported to inhibit PG synthesis. The established effectiveness of indomethacin and aspirin were used for reference in testing the following drugs: naproxen, mefenamic acid, tranylcypromine, and phenelzine. Only naproxen was found to be effective, but since it was no more potent than indomethacin, the latter drug was used for subsequent work. Indomethacin was stereotaxically implanted into several hypothalamic regions known to regulate TSH secretion, and sequential plasma samples were analyzed for TSH by radioimmunoassay. Bilateral implants of indomethacin in the anterior hypothalamic area increased TSH secretion throughout the 72 hr period of study. Sham inplants at this site and indomethacin implants in other nearby sites were ineffective. These findings suggest that endogenous PGs play an inhibitory role in the hypothalamic regulation of pituitary secretion.

Over the past couple of decades it has become well recognized that the prostaglandins (PGs) can exert a wide variety of endocrine and metabolic effects. In particular, these ubiquitous fatty acids have recently been shown to affect the secretion of each of the hormones of the anterior pituitary gland (*1, 2*). In some instances, the effects of the PGs are direct ones in that they affect the pituitary itself. In contrast, others of the known effects are indirect in that they are exerted at the hypothalamus on factors which in turn alter pituitary secretion.

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This presentation will address only one of the pituitary hormones, thyrotropin or thyroid stimulating hormone (TSH). There is by now considerable evidence from *in vitro* and *in vivo* experiments that certain PGs can enhance the secretion of TSH via a direct pituitary effect (*3-6*). In spite of some rather small differences among the results just cited, most of the available data indicate that the direct stimulation of TSH secretion by the PGs alone is relatively inconsequential. Instead, the most striking effect of the PGs is their ability to enhance pituitary responsiveness to thyrotropin releasing hormone (TRH). This is illustrated in the *in vivo* results presented in Figure 1. In these experiments, various PGs and TRH (or appropriate vehicles) were infused sequentially directly into the anterior pituitaries of pentobarbital-anesthetized female rats, and plasma samples were assayed for TSH by radioimmunoassay.

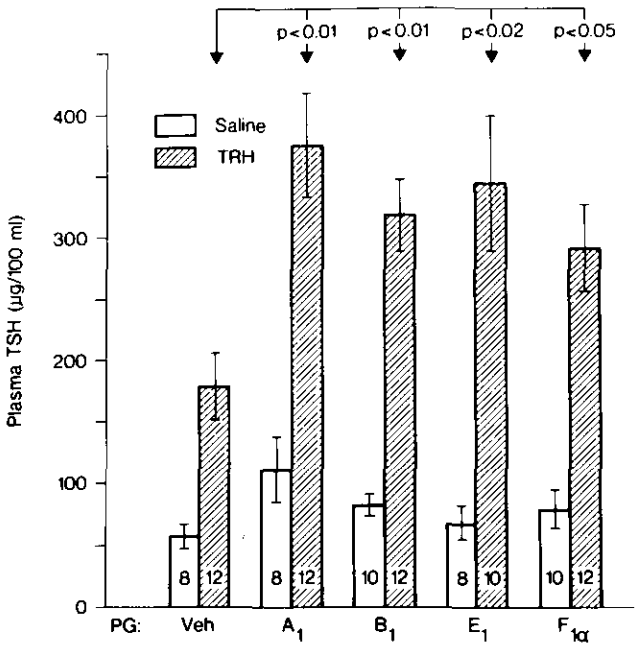

FIGURE 1. Potentiating effects of PGs on TRH-induced TSH secretion. The 1 min PG infusion (2 µg/2µl) was begun at time zero, the 30 sec TRH infusion (1 ng/1 µl) was begun at time 1.5 min, and blood samples were obtained at time 15 min. None of the PG-saline values differs significantly from the control. Reproduced with permission from Brown and Hedge (*4*).

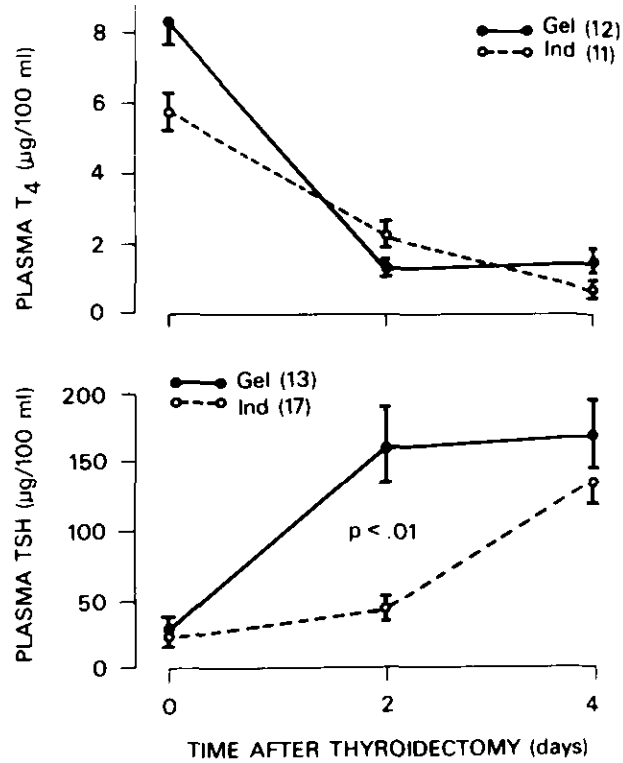

FIGURE 2. Plasma TSH and T_4 levels following thyroidectomy and indomethacin (Ind) or gelatin (GEL) treatment. Reproduced with permission from Thompson and Hedge (*1*).

Having seen that exogenous PGs can affect TSH secretion, the next logical question to pose was whether or not endogenous PGs of the anterior pituitary do affect TSH secretion. One approach to this problem is to assess the secretion of TSH under conditions in which the availability of pituitary PGs has been limited by pharmacological blockade of PG synthesis. The diminution of TSH secretion by such treatment would provide evidence that the endogenous PGs play some role in modulating normal TSH secretion. Sundberg et al. (*5*) have provided some such evidence from *in vitro* experiments, and we have reported results of *in vivo* experiments that support this hypothesis (*7*). For example, Figure 2 demonstrates that indomethacin (three subcutaneous injections of 3 mg each during the first 24 hr) virtually abolishes the compensatory increase in plasma TSH levels following surgical thyroidectomy. Similar results are seen when using aspirin instead of the indomethacin, but higher doses are required. It is also worth noting that the normal rise in TSH is seen after the effect of the indomethacin disappears, indicating that the effect is not due to some type of irreversible tissue damage.

In contrast to the well documented pituitary effects of PGs regarding TSH secretion, only few studies have investigated the possibility of an indirect effect at some suprapituitary site. Our own studies (*4*) and those of others (*8*) have indicated that exogenous PGs injected into the medial basal hypothalamus or lateral cerebral ventricles respectively fail to alter plasma TSH levels. It was clear from these studies that there is no significant effect of any of several prostaglandins even at a dose that is 10-40 times greater than the dose of PGE_1 which when injected in the same way will stimulate the secretion of ACTH (*9*, *10*). We were prepared to accept the likelihood that PGs at the hypothalamic level do not increase TSH secretion. However, the fact that the mean TSH levels in all PG-treated groups were numerically (though not significantly) less than controls at least suggested that the PGs might have an inhibitory effect which could not be demonstrated optimally in the lower (i.e., basal) portion of the secretory range. We therefore wanted to design experiments which might be more appropriate to demonstrate such an inhibitory effect if it exists. First, we proposed to test the effects of PGs under conditions in which the hypothalamus is actively stimulating TRH. In theory, this can be achieved by exposure to a cold environment, by electrical stimulation of the hypothalamus, or by

insulin-induced hypoglycemia. While theoretically sound, these approachs have led to a number of practical problems such that we have favored a different (and more successful) approach. If hypothalamic PGs inhibit TSH secretion, then inhibition of the synthesis of PGs would be expected to increase TSH secretion. Such a result should be easier to detect than the suppression of basal TSH levels. By now, this pharmacological approach is quite common in both *in vitro* and *in vivo* PG studies, and it is the results of some of our preliminary work of this sort that we will present here.

Elsewhere in this issue we have presented results of experiments with enzymatically dispersed pituitary cells *in vitro* that suggest that PGs increase pituitary TSH secretion by acting at the anterior pituitary. Those studies also made use of pharmacological tools to demonstrate that endogenous PGs play such a role. The technique we used in that work offers numerous advantages in studying the regulation of the pituitary's secretion, and the dynamics of its responses. However, the technique is not as easily applicable to a study of the regulation of the secretion of the hypothalamic hormones which in turn regulate pituitary TSH secretion. First, the cells of the hypothalamus, being neurons, cannot easily be dispersed enzymatically. The alternative, incubating fragments of hypothalamic tissue, is subject to all of the standard criticisms related to the exchange of nutrients and metabolites between medium and internal regions of tissue. Other investigators (*11*) have been successful in superfusing hypothalamic tissue "up stream" from dispersed cells in studying ACTH secretion. However, our initial attempts to use this arrangement for our studies of TSH have resulted in very erratic and short-lived release of the hypothalamic factors regulating TSH secretion. Because of this, we have chosen to investigate the possible roles of hypothalamic PGs in the regulation of TSH using an *in vivo* approach.

Indomethacin and aspirin are two of the more common drugs used as experimental inhibitors of PG synthesis, and we have found that both of these drugs can inhibit TSH secretion by acting directly at the pituitary (*7*). However, since the time that we did those experiments, numerous other drugs—in particular nonsteroidal antiinflammatory agents—have also been shown to inhibit PG synthesis in various biological systems (*12, 13*). Thus, it was of interest to see if one or more of these drugs might be significantly better than the indomethacin as an experimental tool in our studies of hypothalamic regulation of TSH secretion. We began by testing selected drugs for their inhibition of thyroidectomy-induced TSH secretion. This rather simple protocol was very appropriate for screening, and it very clearly showed the effects of indomethacin and aspirin in our previous studies. Based on the known potencies relative to indomethacin in other systems, we chose the following drugs at the doses indicated in the legend of Figure 3: naproxen (Naprosyn), mefenamic acid (Ponstel), tranylcypromine sulfate (Parnate), phenelzine sulfate (Nardil).

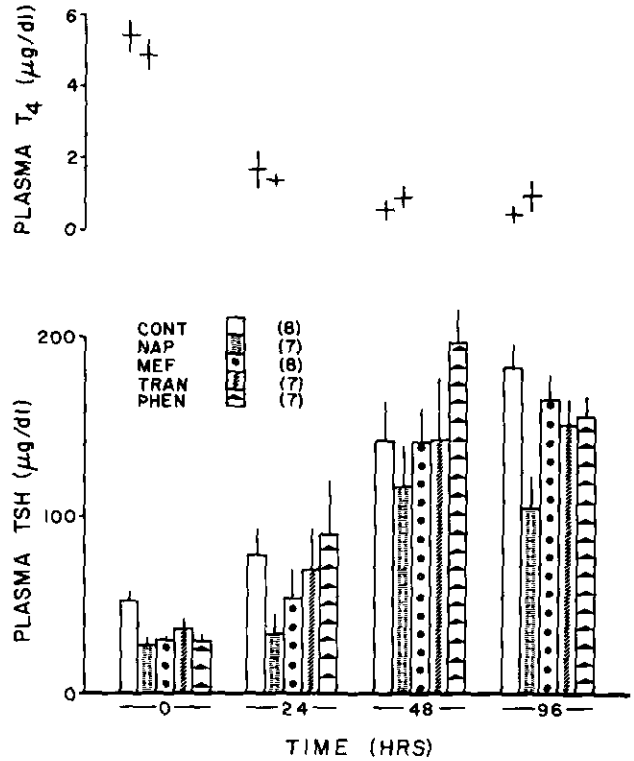

FIGURE 3. Plasma TSH and T_4 concentrations in thyroidectomized rats treated with selected drugs known to inhibit PG synthesis. Drugs and doses (per 100 g body weight) are as follows: CONT, control injections of gelatin solution; NAP, 15 mg naproxen; MEF, 7.5 mg mefenamic acid; TRAN, 1.5 mg tranylcyromine sulfate; and PHEN, 0.015 mg phenelzine sulfate. Thyroidectomy occurred at time zero.

Female rats were thyroidectomized under ether and injected sc at the indicated doses three times over a 24 hr period as in the case of the earlier studies with indomethacin and aspirin. At the times indicated in Figure 3, blood samples were obtained from tail veins under light ether anesthesia for subsequent radioimmunoassay for TSH and T_4. As seen in the lower panel of Figure 3, only naproxen inhibited thyroidectomy-induced TSH secretion ($p < 0.05$ at 24 hr, and $p < 0.01$ at 96 hr). Each of the other drugs tested in this series was without effect.

Before concluding that naproxen blocks TSH secretion directly, it was necessary to show that

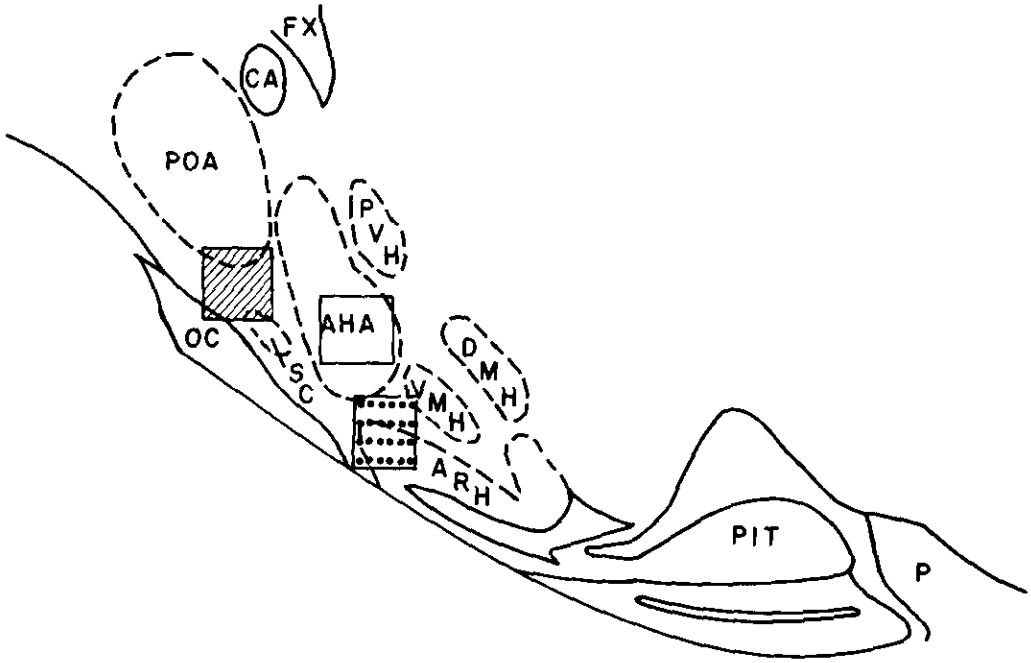

FIGURE 4. Schematic parasagittal section of rat hypothalamus and pituitary with three squares superimposed at sites at which stereotaxic implants were made. Bilateral indomethacin implants were effective only in the region of the open square in AHA. Abbreviations are as follows: FX, fornix; CA, anterior commissure; POA, preoptic area; PVH, paraventricular nucleus; OC, optic chiasma; SC, suprachiasmatic nucleus; AHA, anterior hypothalamic area; DMH, dorsomedial nucleus; VMH, ventromedial nucleus, ARH, arcuate nucleus; PIT, pituitary; and P, pons.

the observed effect is not secondary to some change in the rate at which $T_4$is cleared after thyroidectomy. That is, it was possible that the drug could increase the half-life of T_4 resulting in plasma T_4 levels that were higher than control during the treatment and thus the lower TSH level might be due to the normal function of the negative feedback mechanism. This possibility is ruled out by the T_4 data shown above the TSH data for the control and naproxen groups at all times studied (Figure 3). Even though naproxen has this inhibitory effect, it is clearly no better than indomethacin in this regard. Thus, we decided to use indomethacin in our subsequent study of the possible involvement of hypothalamic PGs in regulating TSH secretion.

The systemic administration of PGs or drugs that alter PG economy is acceptable for screening studies, but given the ubiquitous distribution of the PGs, such a route of injection could result in numerous side effects and thus yield data which are virtually uninterpretable. With this in mind, we chose to test for hypothalamic effects of indomethacin by stereotaxically implanting small amounts of it (45 μg, or talc as control) into specific areas of the hypothalamus known to be involved in the regulation of TRH and TSH secretion. In these studies, it was imperative that we administer the indomethacin locally since we already knew that the drug could decrease pituitary responsiveness to TRH, and we had to use plasma TSH as an endpoint in such studies. These implants were made in pentobarbital-anesthetized (4.5 mg/100 g body weight) female rats. Subsequent blood samples were obtained by cardiac puncture under light ether anesthesia.

Figure 4 depicts diagramatically the areas of interest in this experiment, and it indicates the responsive and the unresponsive regions. Pilot experiments involving single pellets on the midline suggested that such implants might increase TSH secretion, but the differences were not statistically significant. However, when bilateral implants were placed in the anterior hypothalamic area (centered 0.6 mm to each side of midline), significant increases in TSH secretion were observed at all times studied (Fig. 5). Control implants of talc pellets had no such effect. As would be expected the absolute TSH concentrations at time zero (before implantation) in the two groups did not differ. Identical implants of indomethacin placed approximately one mm anterior to this region (Fig. 4, cross-hatched area) were without effect. Somewhat to our surprise, such implants in the medial basal hypothalamus (Fig. 4, stippled area) were also without effect. Although the latter region is definitely involved in

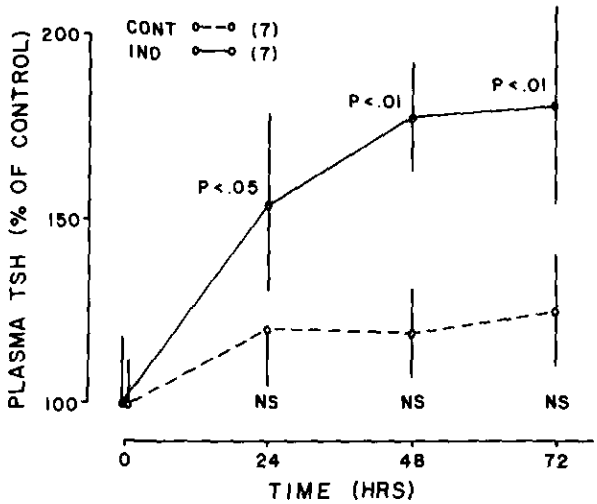

FIGURE 5. Time course of stimulatory effect of indomethacin (Ind) on plasma TSH levels. Bilateral implants of Ind were made in the anterior hypothalamic area of the hypothalamus at time zero. Statistical comparisons (paired *t* test) refer to the time zero control samples taken just prior to implantation.

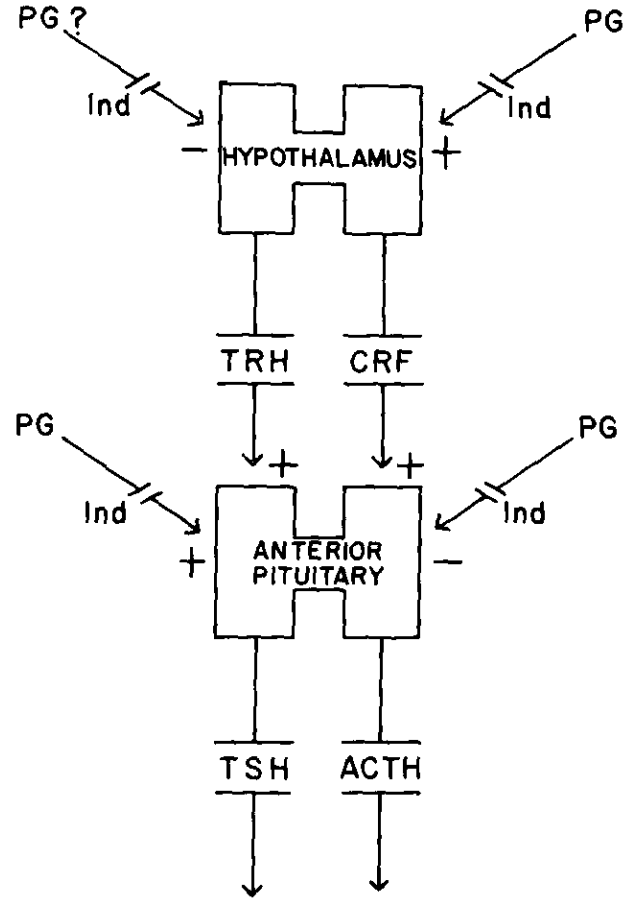

FIGURE 6. Diagram illustrating multiple effects of PGs on TSH and ACTH secretion at both pituitary and hypothalamic levels. A plus denotes stimulation, and a minus denotes inhibition. Broken arrows signify blockade of the PG effect by indomethacin (Ind).

regulating TSH secretion, this result may be due to the fact that this region primarily contains fibers rather than cell bodies.

The results of our earlier pharmacological studies showing that indomethacin decreases TSH secretion in response to stimulation by either TRH or the removal of thyroid hormone feedback suggest that anterior pituitary PGs enhance TSH secretion. In contrast, the present results suggest that PGs of the hypothalamus decrease TSH (presumably via increasing TRH). These findings provide an interesting contrast to the case of ACTH where it is known that indomethacin in the hypothalamus decreases stress-induced ACTH secretion (*14*), whereas indomethacin in the anterior pituitary increases ACTH responsiveness to a CRF preparation (*15*). In the latter case, it has also been shown that the effect of indomethacin was due to its limiting the availability of PGs since exogenous PGs completely reversed this effect of the drug.

The overall picture that emerges regarding the hypothalamic and pituitary effects of PGs on TSH and ACTH secretion is depicted in Figure 6. In the case of each of these tropic hormones, the effect of the PGs at the pituitary is of opposite polarity to the suprapituitary effect. It can also be seen that at each anatomical level the effect of the PGs on TSH is the opposite of the one on ACTH. Of the four PG effects depected on Figure 6, all except the hypothalamic inhibition of TRH-TSH are now supported both by studies with exogenous PGs and with indomethacin or other PG synthetase inhibitors. As far as we are aware, the present study with indomethacin is the only one to have investigated this hypothalamic involvement of PGs in TSH secretion.

This reciprocity is reminiscent of the "inverse relationship" often described regarding the regulation of these two pituitary hormones (*16*). Although this interaction was originally thought to be a pituitary phenomenon, it has been suggested more recently that the interaction may occur above the pituitary. It is interesting to speculate that the reciprocal effects of the prostaglandins could participate in mediating such a relationship at either or both of these levels.

The results of the present study are consistent with the notion that some prostanoid can inhibit TSH secretion by the pituitary indirectly by acting in the region of the anterior hypothalamus. However, to be accepted as firm evidence for this notion,

the effect of the indomethacin would have to be known to be due to its inhibition of PG synthesis, rather than to any of a host of other potential effects of the drug. This distinction could be made by experiments demonstrating that the addition of exogenous PGs will reverse the effect of the drug. Another aspect of the present work that requires further attention concerns the fact that indomethacin blocks synthesis at a very early step (the cyclooxygenase step), and thus prevents the production of virtually all members of the prostaglandin family. Although there was good reason to begin this work with the pharmacological approach, the results obviously do not provide the identity of the specific prostaglandin(s) that normally plays this inhibitory role in the hypothalamus. However, this information could also emanate from the experiments just proposed, and would provide an important confirmation of our interpretation of our present results.

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