Catecholamines and pituitary prolactin release

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Introduction

The secretion of trophic hormones by the anterior pituitary is largely controlled by hypothalamic hypophysiotrophic hormones reaching the gland via the specialized hypophysial portal vasculature. For each of the pituitary hormones, there is a postulated releasing and/or inhibiting hormone. At present, the chemical structure of only three of these compounds (TRH, LHRH and somatostatin), all of which are small peptides, has been established.

Catecholamines occupy a unique position among the various agents associated with homeostasis, since they function both as neurotransmitters in the nervous system, and as hormones in the endocrine system. In fact, catecholamines serve as classical examples of compounds involved in neuroendocrine interactions, and their intimate association with endocrine processes, particularly with reproduction, has been known for decades.

Although much evidence suggests that catecholamines participate in the regulation of secretion of all trophic hormones, their best documented function is the inhibition of pituitary prolactin secretion. The purpose of this presentation is to review the data which establish that dopamine of hypothalamic origin inhibits the secretion of prolactin and to address the question whether dopamine itself functions as a physiological prolactin inhibiting factor (PIF).

Hypothalamic inhibition of prolactin secretion

The evidence for hypothalamic inhibition over the secretion of prolactin is well documented. Hypothalamic lesions or disconnection of the pituitary from the brain, either by stalk section (Diefenbach, Carmel, Frantz & Ferin, 1976; Kanematsu & Sawyer, 1973) or by transplantation of the pituitaries to other sites in the body (Everett, 1954), result in the cessation of secretion of all trophic hormones while that of prolactin increases. Pituitary glands incubated in vitro release copious amounts of prolactin, but only minute amounts of other hormones. The reverse occurs when hypothalamic extracts are added to incubated pituitaries (Talwalker, Ratner & Meites, 1963), resulting in a dose-related increase in the secretion of LH, FSH, TSH, etc., concomitant with a proportional reduction in the release of prolactin. Similarly, injection of hypothalamic extracts into experimental animals, either systemically (Amenomori & Meites, 1970) or into hypophysial portal vessels (Porter, Mical, Ben-Jonathan & Ondo, 1973), reduces circulating prolactin levels but increases those of other hormones.

As the neurohumoral hypothesis developed (Green & Harris, 1947; Scharrer & Scharrer, 1954), it was postulated that the hypothalamus contains a factor, PIF, which inhibits the secretion of prolactin, and several releasing hormones, each of which stimulates the release of the corresponding trophic hormone. In the absence of a specific target gland hormone to feedback on the pituitary, the control mechanism for the secretion of prolactin seemed relatively simple. It was therefore suggested that all factors able to modify the secretion of prolactin do so by way of
changing the hypothalamic content and secretion rates of the postulated PIF. However, it is recognized today that such a model was over-simplified and inadequate. For example, very strong evidence indicates that, in addition to PIF activity, the hypothalamus of several species contains a prolactin releasing activity (PRF) (Nicoll, Fiorindo, McKenney & Parsons, 1970; Valverde, Chieffo & Reichlin, 1972). TRH, originally thought to be specific for the release of TSH, also stimulates the secretion of prolactin (Bowers, Friesen, Hwang, Guyda & Folkers, 1971). Indolamines stimulate significant release of prolactin (Kamberi, Mical & Porter, 1971; MacIndoe & Turkington, 1973) and oestradiol acts both in vivo (Bridges & Goldman, 1975) and in vitro (Nicoll & Meites, 1962; Vician, Shupnik & Gorski, 1979) to augment prolactin secretion. Several other compounds, such as GABA (Pass & Ondo, 1977), enkephalins (Lien, Fenichel, Garsky, Sarantakis & Grant, 1976) and vasoactive intestinal peptide (Kato et al., 1978), have been shown to modify prolactin secretion. Although some of these effects might be due to pharmacological rather than physiological manifestations, the complex nature of the control of prolactin secretion should be borne in mind.

The ability of catecholamines, and in particular dopamine, to inhibit the secretion of prolactin has been known for a decade. Initially, most of the data were derived from pharmacological studies in which the effects of adrenergic drugs on the secretion of prolactin were evaluated. Indeed, a wealth of information exists to indicate that drugs which increase brain catecholamine levels, by enhancing their synthesis or by blocking their degradation, significantly depress serum prolactin levels. On the other hand, drugs known to deplete hypothalamic catecholamines or to block their receptors concomitantly cause elevation in serum prolactin levels (for review see Weiner & Ganong, 1978).

The pharmacological studies were supported by physiological investigations in which the amines themselves or their precursors were introduced directly into the brain or into the systemic circulation. Administration of dopamine into the third ventricle of rats (Kamberi & Porter, 1971; Ojeda, Harms & McCann, 1974) caused marked reduction in plasma prolactin whereas epinephrine and norepinephrine were less effective. Systemic administration of the catecholamine precursor L-dopa into patients suffering from Forbes Albright syndrome (Turkington, 1972), monkeys after pituitary stalk section (Diefenbach et al., 1976) or rats with complete lesions of the median eminence (Donoso, Bishop & McCann, 1973) resulted in suppression of plasma prolactin levels. Injection of L-dopa into rats bearing anterior pituitary transplants under kidney capsule (Donoso, Banzan & Barcagioni, 1974) caused an immediate fall in plasma prolactin concentration. Pretreatment with a dopa decarboxylase inhibitor, however, prevented this fall, suggesting that L-dopa influenced prolactin secretion after being converted to dopamine.

These studies, and many more, have led to the formulation of the two hypotheses currently being debated. One implies that dopamine stimulates the release of a hypothalamic PIF, which in turn inhibits the secretion of prolactin, whereas the second suggests that dopamine itself is a physiological PIF. The following discussion will address this question by examining selected studies utilizing in-vivo and in-vitro approaches.

Prolactin inhibiting activity in the brain

Although much is known about the physiology of prolactin, i.e. when and in what circumstances there are alterations in its release (for reviews see, Meites et al., 1972; Nicoll, 1974), surprisingly little is known about the chemical nature or the distribution of the compound(s) responsible for the control of its secretion.

The purification, isolation and eventual synthesis of the first releasing hormone, TRH (a tripeptide), in 1969 marked a turning point in the field of neuroendocrinology (for reviews see, Guillemin, 1978; Schally, 1978). This discovery was followed by the structural elucidation and synthesis of the decapeptide, LHRH, and the tetra-decapeptide, somatostatin. However, the
chemical nature of a hypothalamic PIF remains unknown, and not necessarily because of neglect. In spite of access to hundreds of thousands of animal hypothalami and specialization in elaborate isolation and purification techniques, workers in several laboratories have failed so far to establish the chemical identity of PIF. There might be several reasons for this lack of success. First, PIF may not be a small peptide like the releasing/inhibiting hormones isolated to date, and therefore techniques for peptide chemistry may not be adequate. Second, PIF may be a labile compound that does not withstand extensive purification procedures. Third, there might be more than one compound with a prolactin release inhibiting activity in hypothalamic extracts. Indeed, the reports on the isolation of PIF over the past few years have been conflicting, confusing, and inconclusive. For example, Schally, Arimura, Takahara, Redding & Dupont (1974) reported that, following Sephadex chromatography of hypothalamic extracts, several fractions with PIF activity were eluted from the column, one of which had a high concentration of catecholamines. Later, however, Dupont & Redding (1975) reported that they had purified a fraction with PIF activity from porcine hypothalami which had different physico-chemical characteristics from dopamine and norepinephrine. More recently, Schally, Redding, Arimura, Dupont & Linthicum (1977) identified the PIF activity in their fraction as gamma-amino butyric acid. In the latest report from the same laboratory, Schally et al. (1979) maintain that both PIF and PRF might be peptides after all.

In parallel with the thrust to isolate the releasing/inhibiting hormones and study their physiology, efforts were undertaken to determine their distribution in the brain. Utilizing immunological, immunocytochemical and microdissection techniques, most researchers agree that the highest concentration of hypophysiotrophic hormones is found in the median eminence in the vicinity of the primary capillary bed of the hypophysial portal vessels (for reviews see Brownstein, 1977; Zimmerman, 1977). However, these hormones are not confined only to the hypothalamus, as was the general concensus several years ago, but display a wide extrahypothalamic distribution (Oliver, Eskay, Ben-Jonathan & Porter, 1974; Hökfelt et al., 1975).

In view of the above evidence, it is of interest to note that a marked resemblance exists between the distribution of the hypophysiotrophic hormones and that of catecholamines. Utilizing fluorescent histochemical methods, Fuxe (1965) and Björklund, Moore, Nobin & Stenevi (1973) identified the tubero-infundibular dopaminergic pathway, whose cell bodies are located in the arcuate and paraventricular hypothalamic nuclei, and whose axon terminals are adjacent to the primary capillary plexus of the hypophysial portal vessels. These findings were supported by Kavanagh & Weisz (1974) using a fluorometric method and by Kizer, Palkovits, Tappaz, Keibabian & Brownstein (1976) utilizing a combination of microdissection and radioenzymic assay; both groups found the highest concentration of catecholamines in the pituitary stalk–median eminence region.

A comparison between the subcellular compartmentalization of TRH, LHRH, dopamine and norepinephrine has also been made. Fractionation of hypothalamic homogenates on continuous sucrose density gradients resulted in a similar distribution of TRH, LHRH and the catecholamines. On the basis of sedimentation characteristics as well as susceptibility to detergent treatment and hypo-osmotic shock, Barnea, Ben-Jonathan, Colston, Johnston & Porter (1975) and Barnea, Ben-Jonathan & Porter (1976) concluded that catecholamines, TRH and possibly LHRH are contained in nerve endings, the synaptosomes.

Hypophysial portal blood as the neurovascular link

The anterior pituitary of most mammals receives blood through portal vessels only (for reviews, see Porter et al., 1973; Bergland & Page, 1979). Although it is recognized today that the hypophysial vasculature is more complex than was previously thought, for the purpose of simplicity it can be stated that there are two divisions to the portal vasculature. One is derived
from the superior hypophysial artery, has its primary capillaries in the median eminence, and is connected to the pituitary via the long portal vessels which lie on the surface of the stalk. The second is located in the infundibular process (neurohypophysis), derives its origin from the middle and inferior hypophysial arteries, and has a common capillary bed with numerous connections with the adenohypophysis.

The unique arrangement of this vasculature, which is remarkably suitable for exchange of substances between the hypothalamus and the anterior pituitary, and the absence of direct innervation of the adenohypophysis provided the basis for the neurohumoral hypothesis. One of the underlying assumptions of this hypothesis was that hypothalamic substances regulating the secretion of pituitary trophic hormones reach the gland via the hypophysial portal vasculature. Therefore, it was expected that: (a) the presence of releasing/inhibiting hormones in portal blood should be demonstrable, (b) their concentrations in portal blood should be higher than in the systemic blood, and (c) there should be variations in their concentrations in portal blood which would reflect alterations in the secretion rates of the corresponding trophic hormones.

Before the introduction of radioimmunoassays for hypophysiotrophic hormones, the only method for assessing their presence in portal blood was by bioassays, which were cumbersome, but nonetheless essential. In 1973, Ben-Jonathan, Mical & Porter developed a procedure in which a hemipituitary was placed in a chamber and superfused with hypophysial portal blood collected from an anaesthetized rat. The contralateral hemipituitary was simultaneously superfused with blood from a femoral artery, thus serving as a control. The secretion rates of LH or prolactin in the effluence of the portal blood-superfused hemipituitary was used to evaluate the secretion rates of LHRH or PIF by the hypothalamus. By using this combined in-vivo-in-vitro approach, an elevated LHRH activity in portal blood from ovariectomized rats was apparent, but little or no LHRH activity was evident in portal blood from dioestrous rats. However, when the effluent blood from the same experiment was analysed for prolactin (N. Ben-Jonathan, unpublished observations), there was little evidence for PIF activity in portal blood, possibly because the high concentration of prolactin itself in portal blood was masking any PIF activity.

Indeed, the demonstration of PIF activity in portal blood as judged by biological criteria, is yet an unresolved question. Kamberi, Mical & Porter (1970) were unable to show a difference in PIF activity between portal and arterial blood from male rats when tested with hemipituitaries incubated in vitro. However, portal plasma collected from rats after intraventricular injection of dopamine exhibited a significant PIF activity. These results could be interpreted in two ways: either that dopamine caused an increased secretion of PIF into portal blood, or that dopamine itself passed from CSF into portal blood and caused the inhibition of prolactin secretion. Kamberi & Porter (1971) ruled out the second possibility, based on their findings that infusion of dopamine into single portal vessels in vivo did not result in the inhibition of prolactin secretion. On the other hand, Takahara, Arimura & Schally (1974) reported that when dopamine was dissolved in 5% glucose rather than in saline it had PIF activity when infused into a single portal vessel. The reason for this discrepancy may be that the glucose-containing solution prevented the rapid spontaneous oxidation of dopamine which might have occurred in the saline solution.

The opportunity to search for dopamine in hypophysial portal blood presented itself after the development of a sensitive double-isotope radioenzymic assay (Ben-Jonathan & Porter, 1976), which is capable of measuring simultaneously dopamine, norepinephrine and epinephrine in as little as 25–50 µl of plasma. By utilizing this assay, Ben-Jonathan, Oliver, Weiner, Mical & Porter (1977) reported that the concentration of dopamine, but not that of norepinephrine or epinephrine, was significantly higher in hypophysial portal than in arterial blood from the same rats. Moreover, at times of elevated prolactin secretion, such as during pro-oestrus, dopamine level in portal blood was low, whereas it was elevated during oestrus and dioestrus when plasma prolactin levels in the rat are low. Thus, this observation provided critical evidence that dopamine is released by the hypothalamus into the portal vasculature and therefore could act
directly at the pituitary level to inhibit prolactin release. Similar results were later reported by other investigators using either a radioenzymic assay (Cramer, Parker & Porter, 1979) or a liquid chromatographic-electrochemical assay (Gibbs & Neill, 1978) for measuring dopamine in portal blood.

The interrelationship between dopamine and prolactin during the transition time between late gestation (low prolactin secretion) and early lactation (elevated prolactin secretion) was also investigated (Ben-Jonathan, Neill, Arborgast, Peters & Hoefer, 1980). As can be seen in Text-fig. 1, dopamine levels in portal blood during late gestation were significantly higher than those during lactation, suggesting that the suppression of prolactin release during this stage of pregnancy might be caused by increased hypothalamic dopamine release. However, prolactin elevation on the last day of gestation was not associated with significant reduction in hypothalamic dopamine secretion. Therefore, it is possible that this transient rise in prolactin, which is attributable to oestradiol (Bridges & Goldman, 1975), occurs as a result of direct stimulation of pituitary lactotrophs by oestradiol (Vician et al., 1979) rather than by its action via the dopaminergic system.

Text-fig. 1. Concentrations (mean ± s.e.m. for no. of observations indicated) of dopamine in hypophysial portal blood and prolactin in the systemic circulation during late pregnancy and lactation in rats. Prolactin was determined by RIA in trunk blood collected by decapitation. Dopamine was determined by radioenzymic assay in blood collected from pentobarbital-anaesthetized rats.

Removal of the young from the mothers for 24 h (Text-fig. 1), which resulted in a marked suppression of plasma prolactin, was also associated with a significant elevation in portal plasma dopamine levels. Separation of the young was not, however, associated with changes in catecholamine concentrations in the hypothalamus or the posterior or anterior pituitaries (Ben-Jonathan et al., 1980).

Although the above studies demonstrate that under several physiological conditions of low prolactin secretion hypothalamic dopamine release is elevated and vice versa, several questions remain. For example, reciprocal relationship between portal blood dopamine and prolactin in the
systemic circulation in the same animal is not evident, which might be due to direct inhibition of pentobarbital anaesthesia on pituitary lactotrophs. Moreover, male rats or castrated female rats have low circulating prolactin as well as low portal blood dopamine concentrations (Ben-Jonathan et al., 1977). Hence, gonadal steroids and/or differences in the sensitivity of pituitary lactotrophs to oncoming dopamine might be involved in the control of pituitary prolactin secretion.

The posterior pituitary and the control of prolactin secretion

The presence of high levels of dopamine in hypophysial portal blood raises an intriguing question: Is dopamine secreted into portal blood from dopaminergic neurones located in the median eminence, or is it diffusing out during the procedure of portal blood collection from the cut axons located in the pituitary stalk which terminate in the posterior lobe? The implications of this enigma are of great interest. If indeed dopamine regulates the secretion of prolactin, it might do so via the posterior pituitary rather than through secretion into the long portal vessels of the median eminence. Therefore, such a route could provide the first evidence for a functional relationship between the posterior and anterior lobes of the pituitary.

Although the two lobes of the pituitary are juxtaposed, little or no evidence is available to support the belief that the posterior pituitary participates in the regulation of trophic hormone secretion. The posterior lobe is a neural tissue which develops during embryonic life from the infundibulum of the base of the brain, whereas the anterior lobe originates from an outgrowth from the roof of the mouth and is therefore not a neuronal tissue. Indeed, the posterior lobe is richly innervated while there is no evidence for direct innervation of the anterior lobe. Furthermore, the anterior pituitary produces its trophic hormones in situ, and receives signals for their release by hormones reaching the gland via the vasculature. The two posterior pituitary hormones, vasopressin and oxytocin, are not produced locally, but in the perikarya of the neurosecretory neurones of the supraoptico-hypophysial tract (for review, see Sachs, Fawcett, Takabatake & Portanova, 1969), which retain their membrane potential and conduction capabilities.

Striking similarities exist between the synthesis, axoplasmic transport, storage and release of catecholamines and vasopressin. Like vasopressin, catecholamines are synthesized in neuronal cell bodies and travel down the axons associated with secretory granules (for review, see Smith, 1971). Vasopressin as well as catecholamines are apparently bound within the granules to proteins, and upon stimulation, both the active compounds and the binding proteins are released into the circulation (Kirshner & Kirshner, 1971).

A similarity between catecholamines and vasopressin is not sufficient, however, to establish dopamine as a posterior pituitary agent whose function is to control prolactin secretion. For that, several prerequisites have to be fulfilled. First, the presence of dopaminergic neurones leading to the posterior pituitary has to be demonstrated. Second, the presence of dopamine at high concentrations in the posterior lobe should be evident. Third, a means by which dopamine may reach the anterior lobe from the posterior lobe should be feasible. Fourth, and most important, it should be demonstrated that the posterior pituitary plays an important role in regulating the secretion of prolactin by the anterior pituitary gland.

Indeed, there are several observations that support some of the requirements listed above. Björklund et al. (1973) demonstrated that, in addition to innervating the median eminence, dopaminergic terminals could be traced down to the intermediate and posterior lobes of the pituitary. Several investigators (Saavedra, Palkovits, Kizer, Brownstein & Zivin, 1975; Ben-Jonathan et al., 1980) reported that the concentration of dopamine in the posterior lobe is the same as or exceeds, that of the hypothalamus. Moreover, Axelrod, Albers & Clemente (1959) reported that of all the central nervous system regions, the anterior and posterior lobes of the
pituitary contained the highest activity of the enzyme associated with inactivation of catecholamines, catechol-O-methyl transferase (COMT).

It can be visualized that if dopamine from the posterior lobe is involved in regulating pituitary prolactin secretion, it might reach the anterior lobe via the rich network of capillaries connecting the two lobes (Bergland & Page, 1979). Furthermore, if the posterior lobe participates in inhibiting prolactin secretion, then its removal should result in the elevation of circulating prolactin, but not of other pituitary hormones. Such an observation has been reported by Oliver et al. (1977), and is supported by unpublished results of our own. Moreover, as shown in Text-fig. 2, acidic extracts of rat posterior pituitaries exhibited PIF activity when incubated with dispersed rat anterior pituitary cells (Hoefer, Arbogast & Ben-Jonathan, 1979). This inhibition was not due, however, to vasopressin and oxytocin which were ineffective in modulating prolactin secretion in all doses tested. Similar results were reported earlier by Nicoll et al. (1970).

**Text-fig. 2.** Inhibition of prolactin secretion from dispersed rat anterior pituitary cells by HCl-extracted rat posterior pituitaries. Values are mean ± s.e.m. for 4 determinations.

It should be emphasized that the role postulated here for the posterior pituitary in regulating the secretion of prolactin is not in disagreement with the general consensus of hypothalamic inhibition over the secretion of prolactin. It is envisioned that if the model suggested here is correct, dopamine is probably only stored, but not synthesized, in the posterior pituitary. By analogy to vasopressin and oxytocin, dopamine is manufactured in neuronal cell bodies located in the hypothalamus and travels down via axoplasmic flow to the posterior lobe where it is stored. Thus, hypothalamic extracts contain sufficient quantities of dopamine to account for the PIF activity. Moreover, elevation of prolactin secretion in the living animal caused by severing the connection between the hypothalamus and the pituitary, i.e. pituitary stalk section or pituitary transplantation, could be interpreted as a result of disconnecting neuronal tracts leading to the posterior lobe rather than by interfering with the transport of PIF from the hypothalamus to the anterior pituitary through the long portal vessels of the median eminence.

**In-vitro studies and dopamine receptors**

Inhibition of prolactin secretion from isolated pituitaries *in vitro* by acidic extracts of hypothalamic tissue was first reported by Pastels (1961) and Talwalker *et al.* (1963). In 1974, Shaar & Clemens suggested that PIF activity in hypothalamic extracts could be accounted for solely by their dopamine content. This was based on their finding that enzymic digestion with monoamine oxidase or adsorption of catecholamines with alumina abolished the PIF activity. This question, however, is still debated since Enjalbert, Priam & Kordon (1977) reported that
not all PIF activity of a hypothalamic synaptosomal preparation was removed by alumina extraction, although dopamine itself was removed. In addition, Greibrokk et al. (1974) and Schally et al. (1977) reported on the presence of PIF activity in purified hypothalamic fractions which were devoided of catecholamines. However, although the hypothalamus may contain several agents which are capable of inhibiting prolactin secretion in vitro, the crucial question is whether any of these reaches the anterior pituitary in vivo under physiological conditions.

Since the advent of the idea that dopamine itself might function as a PIF, workers in several laboratories have confirmed that dopamine at physiological concentrations inhibited prolactin secretion from isolated pituitaries. MacLeod, Fontham & Lehmeyer (1970) and MacLeod & Lehmeyer (1974) demonstrated that both prolactin synthesis, as judged by incorporation of tritiated amino acids, as well as its secretion, were inhibited by dopamine. Moreover, several catecholamine agonists, such as apomorphine (Smalstig, Sawyer & Clemens, 1974), ergocryptine (Pasteels, Darguy, Frerotte & Ectors, 1971; Caron et al., 1978) and lergotrile (Clemens, Smalstig & Shaar, 1975), were also effective in inhibiting prolactin secretion, and their action was blocked by the dopamine antagonists haloperidol and pimozide (MacLeod & Lamberts, 1978). Norepinephrine was also capable of inhibiting prolactin secretion in vitro but at higher doses than those of dopamine (Birge, Jacobs, Hammer & Daughaday, 1970; Shaar & Clemens, 1974; Caron et al., 1978).

With the development of the method for dispersing viable pituitary cells (Vale, Grant, Amoss, Blackwell & Guillemin, 1972), some of the disadvantages of the previously used hemipituitary incubation method have been alleviated. The dispersed cells provide convenience, reproducibility, sensitivity and the capacity to test large numbers of agents in one experiment. Using pituitary cells dispersed with collagenase and hyaluronidase, Hoefer et al. (1979) showed that there was little inhibition of prolactin secretion by dopamine at 24 h after dispersion, but if the length of preincubation was extended to 96 h, dopamine at a concentration of 10^{-7} M caused a 75–80% inhibition. In contrast, the gonadotrophs retained their ability to respond to LHRH-stimulated LH release throughout this period, suggesting that there might be differences in the susceptibility and possibly the structure of the LHRH and the dopamine receptors on the gonadotrophs and lactotrophs, respectively. Hoefer et al. (1979) also demonstrated differences in the sensitivity of cells taken from male, pregnant female or lactating female rats to the various agents modulating prolactin secretion. Therefore, in addition to changes in hypothalamic secretion rates of releasing/inhibiting factors, an additional control mechanism for the secretion of prolactin might be via changes in lactotroph sensitivity to these agents induced by the different physiological states of the animal.

As shown in Text-fig. 3, dopamine inhibited and TRH stimulated prolactin secretion from dispersed pituitary cells in a dose-dependent manner. Moreover, the effective concentrations of these agents are well within those found in hypophysial portal blood (Text-fig. 1) or in hypothalamic tissue (Oliver et al., 1974; Ben-Jonathan & Porter, 1976). An interesting finding is the demonstration of PIF activity in hypothalami extracted with HCl, whereas the perchloric acid extracts had a slight stimulation rather than inhibition. This observation suggests the presence of a PRF activity in hypothalamic extracts which is more potent than its PIF activity since the perchloric acid extract contains as much catecholamines as the HCl extract.

Important support for the direct action of dopamine on the anterior pituitary gland came with the identification of specific dopamine receptors in anterior pituitaries of rats, sheep and cows (Brown, Seeman & Lee, 1976; Creese, Schneider & Snyder, 1977; Cronin, Roberts & Weiner, 1978). The number of binding sites and their affinity for dopamine and its agonists and antagonists resemble those of the caudate nucleus, a region well known for its extensive dopaminergic innervation. Moreover, the competition of the different agents for displacing [3H]- dihydroergocryptine, a potent dopaminergic antagonist, paralleled their ability to inhibit the release of prolactin from cultured dispersed rat pituitary cells (Caron et al., 1978). In addition, agents involved with the secretion of prolactin, such as TRH, oestrogens and catechol-
Effects of dopamine, TRH and hypothalamic extracts on prolactin secretion by dispersed rat anterior pituitary cells. Each point represents a mean of 4 determinations in a typical experiment.

Oestrogens, did not compete with catecholamines on the same receptor sites, suggesting action via a different mechanism (Cronin et al., 1978). However, due to the heterogeneity of the different cell types in the anterior pituitary, it has been impossible so far to demonstrate changes in the dopamine-dependent adenylate cyclase in anterior pituitaries after exposure to dopaminergic agonists and antagonists (Spano, Govani & Trabucchi, 1978).

In spite of the strong evidence indicating the presence of specific dopamine receptors in anterior pituitary homogenates, several questions remain to be solved. For example: (a) are there changes in the affinity and/or number of binding sites of dopamine which correlate with the physiological state of the animal? (b) are the dopamine receptors localized specifically on pituitary lactotrophs? and (c) what is the mechanism leading to the inhibition of prolactin secretion once the receptors have been occupied? These and many more problems await further investigation.

Conclusions

Prolactin secretion by the anterior pituitary gland is largely controlled by a hypothalamic inhibitory substance, PIF, the chemical identity of which is still debatable. The following findings suggest that dopamine of hypothalamic origin serves as a physiological PIF. (1) Dopaminergic pathways have been demonstrated in the median eminence of the hypothalamus and the posterior pituitary gland. (2) Dopamine, its precursors and agonists are able to inhibit prolactin secretion in vivo and in vitro. (3) Dopamine is present in hypophysial portal blood at higher concentration than in the systemic circulation and has reciprocal relationship with prolactin in a variety of endocrinological states. (4) Specific dopaminergic receptors have been demonstrated in the anterior pituitary gland. It is also proposed that dopamine might inhibit prolactin secretion via two possible routes: one through its secretion into the long portal vessels connecting the median eminence with the anterior pituitary gland, and the other through the short portal vessels connecting the posterior and the anterior lobes of the pituitary.

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