Stimulated Vasopressin Synthesis by a Fetal Hypothalamic Factor

Abstract. *The hypothalamo-neurohypophyseal complex (HNC) of the fetal guinea pig shows a dramatic increase in its content of vasopressin and neurophysin between the 40th and 55th days of gestation. The values for radioimmunoassayable hormone and binding protein are at day 40, 2 milliunits and less than 0.1 microgram; and at day 55, about 100 milliunits and 10 micrograms, respectively. Isotope incorporation experiments with organ cultures of the fetal HNC taken prior to the 35th day of gestation added additional confirmation of the inability of the hypothalamic neurosecretory cells to synthesize vasopressin or neurophysin at this time. However, by the 45th day, similar organ cultures show a vigorous incorporation of labeled amino acids into both hormone and binding protein. Furthermore, the HNC of the 45-day-old fetus apparently contains a factor that stimulates specifically the biosynthesis of vasopressin and neurophysin in HNC cultures from the adult guinea pig. This factor is not detectable in either cortex or liver of the 45-day-old fetus or in the fetal HNC taken prior to or after the period of exponential rise (40th to 55th days) of hormone and binding protein.*

An organ culture of the adult guinea pig hypothalamo-neurohypophyseal complex (HNC) capable of the synthesis, transport, and release of vasopressin and its carrier protein, neurophysin, has been described in reports of work performed in our laboratory (*1–4*) and utilized for studies on the biochemical mechanisms of hypothalamic neurosecretion. Simultaneous investigations of the developing HNC led us to the discovery of a factor present in the fetal guinea pig HNC which greatly stimulates the production of radioactive hormone and vasopressin binding protein in adult HNC cultures incubated with ^{3}H-labeled amino acids (*2*).

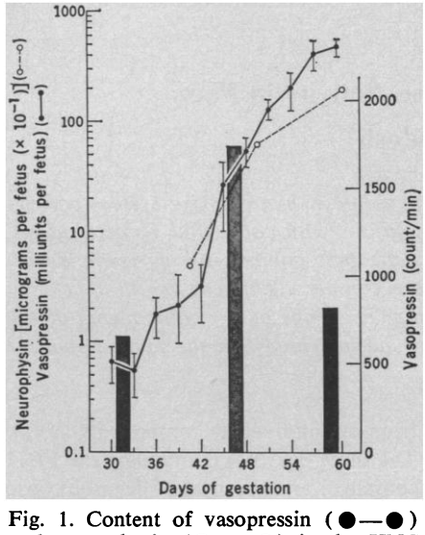

Fig. 1. Content of vasopressin (●—●) and neurophysin (○---○) in the HNC of developing guinea pigs. The bars show the amount of ^{3}H-labeled amino acid incorporated into vasopressin in HNC organ cultures established from adult guinea pigs, which were labeled in medium conditioned by incubation with fetal HNC (one fetal HNC per adult HNC in 1 ml of medium for 24 hours). Each incorporation experiment represents the results from ten pooled organ cultures. Vasopressin content data are shown ± standard error of the mean.

In utero, the fetal HNC of the guinea pig shows a characteristic and striking pattern in the parallel appearance of vasopressin and neurophysin (that is, the secretory components of the mature neurosecretory neuron). Histochemical studies by Donev (*5*) on the developing guinea pig HNC showed that neurosecretory material first becomes evident at the 39th day of gestation. These cytochemical techniques form the classic methodology for detecting aggregates of neurosecretory granules that, in the adult HNC, constitute the major intraneuronal storage form of hormone and binding protein. By means of much more sensitive radioimmunoassay procedures (*2*, *4*), however, we could detect and measure vasopressin and neurophysin in the fetal HNC at about the 30th to the 35th days of gestation. The quantities at this time are, however, extremely low, and it is, in fact, during the 40th to the 55th days of gestation that a dramatic increase in both neurosecretory substances is observed (Fig. 1). At day 40, the respective values for vasopressin and neurophysin in the fetal HNC are 2 munit and less than 0.1 μg, respectively, whereas by the 55th day there has occurred approximately a 100-fold increase in the levels of both components. Although Perks (*6*) has reported that the neural lobe of the fetal seal and sheep contain vasotocin, often considered the ancestral molecule to the neurohypophyseal hormones of vertebrates (*7*), we were unable to detect vasotocin in the fetal HNC (*8*).

The analytic data described above, which outline the onset of a rapid production and storage of vasopressin and neurophysin by the neurosecretory neurons, concurs with isotope incorporation experiments carried out with fetal HNC organ cultures. Thus, it was consistently observed that organ cultures established from fetuses of less than 35 to 40 days of gestation are unable to incorporate radioactive amino acids into either vasopressin or neurophysin within our limits of detectability, whereas the HNC of 45-day-old fetuses showed readily measurable incorporation of labeled precursors (200 to 300 count/min into vasopressin isolated from eight pooled cultures) into both substances under similar conditions of incubation. Furthermore, it was found that these latter cultures contained a factor (or factors) which apparently stimulated specifically the biosynthesis of hormone and binding protein in cultures of the HNC of adult guinea pig.

In the standard experiment, cultures of the adult HNC are established for 7 days [a time previously shown to be optimal for in vitro vasopressin biosynthesis (*2*)] and then incubated in medium containing ^{3}H-labeled amino acids for 24 hours (*1*). Thereafter, acid extracts of the tissues are obtained, and vasopressin and neurophysin are isolated and rigorously purified to constant specific activity (*2*, *4*); the size and specific activity of the amino acid pools are monitored by the fluorescamine procedure (*9*). During this incu-

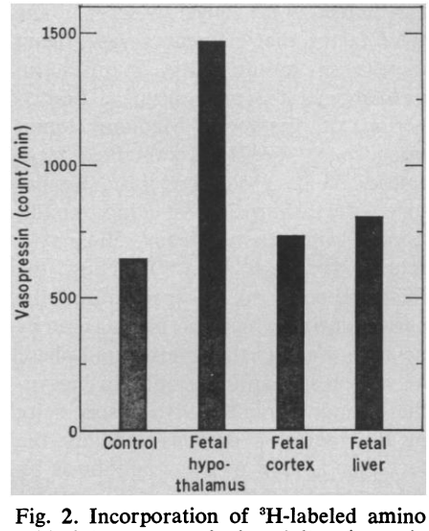

Fig. 2. Incorporation of ^{3}H-labeled amino acids into vasopressin in adult guinea pig HNC organ cultures. The cultures had been established for 7 days and were labeled on day 8 in medium that had been incubated during the previous 24 hours with the indicated fetal tissues taken from fetuses of 45 days gestational age. Each point represents the results of ten pooled cultures. Qualitatively similar data were obtained when adult tissues were treated with extracts of fetal tissues (see text).

bation period labeled amino acids are incorporated into hormone, neurophysin, and many other cellular proteins and polypeptides (*2*, *4*). A two- to fourfold stimulation of vasopressin and neurophysin labeling was observed when the adult HNC cultures were labeled in the presence of 45-day-old fetal HNC tissues. Apparently unaffected was the labeling of acid-soluble (*p*H 1.0) and -insoluble proteins and the free amino acid pool of the adult cultures, as well as vasopressin and neurophysin of the fetal HNC tissues.

The possibility that the fetal HNC was releasing into the medium some factor that stimulates vasopressin and neurophysin biosynthesis in the adult tissue was examined in a number of ways. Adult guinea pig HNC cultured in media that had been conditioned by prior incubation with fetal HNC tissues for 12 to 24 hours also showed an apparent specific two- to fourfold stimulation of labeling of vasopressin and neurophysin in adult cultures (that is, incorporation of ^{3}H-labeled amino acids into other measured protein fractions was not affected significantly). Furthermore, labeled hormone is not found in significant quantities in either control or stimulated labeling media. In these experiments, a clear concentration-response relation, however, could not be demonstrated since serial dilutions of conditioned media were equally effective until a critical dilution is reached beyond which the stimulatory effect is no longer observed. The fetal factor that influences vasopressin metabolism in adult HNC cultures can be demonstrated only during a limited period of gestation. Medium conditioned with the HNC explanted from fetuses 33 days old shows no stimulatory effect, nor does medium conditioned with tissue from 56-day-old fetuses (bars in Fig. 1). Thus, the factor appears to be specific for the period during which the neurosecretory neurons undergo their most rapid phase of vasopressin and neurophysin biosynthesis and storage. Further specificity of the factor is demonstrated by the fact that among media conditioned by either fetal HNC, liver, or cerebral cortex (all from fetuses of 45 days gestation), or adult HNC, only the first stimulates incorporation of ^{3}H-labeled amino acids into vasopressin (Fig. 2).

Further evidence for the presence of a "stimulatory factor" contained within the fetal HNC was obtained by preparing extracts of such fetal tissues. Freshly excised HNC tissues (42 to 47 days of gestation) were homogenized in ice-cold, phosphate-buffered saline and centrifuged at 100,000*g* for 2 hours. Addition of this soluble fetal HNC extract to the standard labeling medium again led to a two- to fourfold stimulation in the incorporation of radioactive amino acids into vasopressin of the adult HNC organ cultures. Again, this stimulation occurs in the absence of significant changes in the incorporation of labeled amino acids into total protein; similar extracts prepared from fetal liver and cortex had no effect. The complexity of the assay for the stimulatory factor (isolation of isotopically pure, labeled vasopressin or neurophysin, or both) has made its physical and biological characterization difficult. Our initial fractionation studies indicate that the factor is excluded from Sephadex G-25, but included in Sephadex G-75, suggesting a molecular weight in the 5,000 to 70,000 range, or alternatively that it is associated with other molecules in this range. Further preliminary studies suggest that the factor is sensitive to the action of trypsin and is thus polypeptide in nature.

The existence of a factor specifically in the fetal hypothalamus, which stimulates synthesis of vasopressin in adult HNC organ cultures, may provide a useful tool for further elucidation of mechanisms involved in the neurosecretory process. The fact that the factor is present only in the gestation period during which the vasopressin neurosecretory system is rapidly developing adds to its interest and may have important implications in the developmental process.

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Absence of Pyruvate Decarboxylase Activity in Man: A Cause of Congenital Lactic Acidosis

Abstract. *A complete deficiency in the pyruvate dehydrogenase system activity contributed to the death of a 6-month-old infant with congenital lactic acidosis. The enzymatic block could be isolated to the first component, pyruvate decarboxylase (E_1) of the pyruvate dehydrogenase complex. This enzymatic deficiency allowed a demonstration of an "intercomplex" exchange of the components of the mammalian pyruvate dehydrogenase system and indicated that the first component is normally present in an apparent excess.*

The clinical syndrome congenital lactic acidosis is not a single disease entity but rather may be caused by a variety of genetically determined enzyme deficiencies including pyruvate carboxylase (E.C. 6.4.1.1) (*1*, *2*), fructose-1,6-diphosphatase (hexose diphosphatase E.C. 3.1.3.11) (*3*), and pyruvate dehydrogenase (lipoate) (E.C. 1.2.4.1).

Pyruvate dehydrogenase (PDH) is a multienzyme system that can be purified and separated into at least three separate components and then reconstituted from the individual components (*4*, *5*). The three enzymes comprising the PDH complex are pyruvate decarboxylase (E_1) (E.C. 4.1.1.1), dihydrolipoyl transacetylase (E_2) (E.C. 2.3.1.12), and dihydrolipoyl dehydrogenase (E_3) (E.C. 1.6.4.3); they exist in a molecular ratio of 12 : 1 : 6 (*4*). Pyruvate decarboxylase in vivo exists in an active and inactive form (*6*, *7*). The conversion from the active to the inactive form is catalyzed by the kinase, pyruvate decarboxylase:ATP transphosphorylase, with the conversion from