Life Sciences, Vol. 52, pp. 2017-2025 Pergamon Press
Printed in the USA

EVIDENCE FOR THE PRESENCE OF NORADRENERGIC NEURONS AND THEIR INHIBITORY ACTION ON LUTEINIZING HORMONE-RELEASING HORMONE RELEASE IN CULTURED FETAL RAT HYPOTHALAMIC CELLS

Micheline Héry*, Anne Marie François-Bellan, Paule Deprez, Maxime Faudon and Francis Héry

Faculté de Médecine Nord, Laboratoire de Neuroendocrinologie Expérimentale, INSERM U 297, Bd P. Dramard, 13916 MARSEILLE Cedex 20 FRANCE

(Received in final form April 8, 1993)

Summary

The control of LHRH release by catecholamine systems during fetal life (embryonic stages) was studied using hypothalamic neurons in primary cell cultures and an attempt was made to characterize the receptor type involved. Phenylephrine and clonidine, respectively α_1 an α_2 adrenoreceptor agonists, both inhibited LHRH release. These agonist inhibitory induced-effects were antagonized by the respective α_1 and α_2 adrenoreceptor antagonists (prazosin and rauwolscine). Both prazosin and rauwolscine applied alone induced a marked increase in LHRH release. Similarly, inhibition of catecholamine synthesis obtained by α-methyl-para-tyrosine (α-MT) led to a significant increase in LHRH release. The stimulatory effects induced by α_1 and α_2 adrenoreceptor antagonists or by α-MT on LHRH release suggest the presence of noradrenergic and/or adrenergic cells in fetal hypothalamic cultures. Therefore, catecholamine contents were measured in fetal hypothalamic cells in culture. Measurable amounts of norepinephrine and dopamine were found in cells, although epinephrine was undetectable. These results show : **1** - noradrenergic cells are present in primary culture of fetal hypothalamic cells. **2** - This intrinsic hypothalamic noradrenergic system exerts an inhibitory control on LHRH release at an early stage of development through α_1 and α_2 adrenoreceptors.

The regulation of luteinizing hormone-releasing hormone (LHRH) synthesis and release is a critical determinent of reproductive function. One primary regulator of LHRH release is norepinephrine (NE) (1,2). The preoptic area which contains most hypophysiotropic LHRH cells is innervated by catecholaminergic afferents that are mainly noradrenergic (3) Although it is not known if LHRH neurons themselves possess adrenergic receptors, activation of α_1 and possibly α_2 receptors with selective agonists stimulates LHRH release (4,5) However there is now a growing body of evidence suggesting that the nature of the LHRH response to adrenoreceptor stimulation is age related and modulated by gonadal steroids (6,7) It has been demonstrated that the catecholaminergic control of LHRH release is predominantly inhibitory in prepubertal rats (7)

Reports on the development of the hypothalamic LHRH neuron system in the rat have shown that such neurons are detected as early as 13 days of fetal life and that they conclude migration to their final position in the septopreoptic diagonal band area on days 16-17.5 of gestation (8). It has been further suggested that LHRH may influence luteinizing hormone (LH) secretion prenatally since the presence of LHRH within the gonadotrophs and the expression of specific

* To whom correspondence should be addressed

0024-3205/93 $6.00 + .00

LHRH receptors in the pituitary gland have been demonstrated at 14 and 15 days of gestation, respectively (9). The hypothalamic catecholaminergic system of 17 day old fetuses appears to be mature enough to participate in the neuroendocrine regulation as neuromodulators (10). This has led us to consider that NE could regulate the release of LHRH during fetal life. The effects of agents that affect catecholaminergic neurotransmission were examined using fetal hypothalamic neurons in primary culture.

The purpose of this work was to address the following questions (a) whether fetal LHRH release can be influenced by catecholaminergic activation ? (b) whether this control is predominantly inhibitory as demonstrated in prepubertal rats ? (c) and which receptor types are involved in this control ?

Methods.

1) CULTURE CONDITIONS

Pregnant Sprague-Dawley rats from 16 days of gestation were anesthetized with pentobarbital and fetuses were removed aseptically. The day of sperm identification was designated as day 0 of gestation.

Primary cell cultures were prepared by mechanically dissociation of the hypothalami in F12-DMEM (50/50) medium supplemented with 10% fetal calf serum, 1 mM glutamine, 0.6% glucose as well as penicillin (50 U/ml) and streptomycin (50 µg/ml). After being resuspended in a serum-free medium, they were seeded at a density of 1.5 x 10^6 cells in 1.5 ml of medium per 35 mm plastic tissue culture dish. Tissue culture dishes were pretreated as follows : they were coated with D-polylysine (10 µg/ml) for 12 h, rinsed with distilled water, incubated for 2 h with 20% fetal calf serum which was then withdrawn to dryness. The synthetic culture medium used was that described by Bottenstein and Sato (11) supplemented with17-ß-estradiol (10^{-12}M), progesterone (2 x 10^{-8} M), triidotyronine (10^{-9} M) and arachidonic acid (1µg/ml) plus docosahexaenoic acid (0.5µg/ml). 17ß estradiol and progesterone improved attachment and survival of cells respectively (12) and did not affect LHRH release at these concentrations. Cultures were maintained at 37°C in 7% CO_2 / 93% air and 92-95% humidity. The medium was first renewed 4 days after seeding and then every 2 days.

2) DETERMINATION OF LHRH CONTENTS IN CULTURED CELLS AND IN FETAL HYPOTHALAMI.

12 days after plating cells, the medium was withdrawn, and cells were extracted in 0.1 N HCl, homogenized, then stored at 4°C for 2 h. Following centrifugation (4000 g, 4°C), the surpernatants were neutralized, frozen, lyophilized and stored at -20°C until LHRH assay. When processed for direct measurement of LHRH contents, fetal hypothalami were homogenized in 0.1N HCl immediatly after dissection and then treated as described above.

3) RELEASE EXPERIMENTS.

Release experiments were performed on day 12 after plating. The medium was withdrawn and cells were incubated for 2 h with 1ml fresh medium. All drugs tested were made up as 50-fold concentrates in medium and added in 20 µl aliquots to 1 ml medium. α-adrenoreceptor agonists were present in the medium during the final 60 min incubation period. Dopamine (DA) receptor antagonists and α- adrenoreceptor antagonists were present during the last 90min incubation period. α-methyl-para-tyrosine (α-MT) was added at the beginning of the incubation period and remained until the end of incubation (120 min). At the end of incubation period, media were collected in polypropylene tubes containing 150 ml RIA buffer (50 mM Na/Na_2 phosphate buffer pH: 7.0 containing 0.9 % (w/v) NaCl 0.1 % (w/v) gelatine). Then media were snap frozen, lyophilized and stored at -20° C until assayed.