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Neurohypophysial hormones of the 1-month-old bovine fetus: absence of vasotocin during mammal development

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The neurohypophysial hormones of the 1-month-old bovine fetus have been identified by their positions in ion-exchange chromatography and their retention times in high-pressure reverse-phase partition chromatography. Arginine vasopressin and oxytocin have been recognized. The molar ratio vasopressin/oxytocin in neurohypophysis is about 6 in the 1-month-old fetus compared with 4 in the 3-month-old fetus, 2.7 in the 7-month-old fetus and 1 in the adult. Vasotocin is virtually absent even in the early fetus (less than 0.1% of arginine vasopressin). The occurrence of a vasotocin gene expressed in the fetus but silent in the adult appears unlikely.

Neurohypophysial hormone; Oxytocin; Vasopressin; Hormone developmental variation; Vasotocin; (Bovine fetus)

1. INTRODUCTION

Mammalian neurohypophysial hormones, vasopressin and oxytocin, are biosynthesized as protein precursors usually processed into a hormonal nonapeptide, a neurophysin and an additional glycopeptide, copeptin, in the case of vasopressin precursor. Cleavages occur during intracellular transport from the rough endoplasmic reticulum to secretory granules stored in neurone endings (reviews [1,2]). Processing is rarely incomplete at the level of neurohypophysis but a partial maturation was observed in guinea pig in which an intermediate encompassing MSEL-neurophysin and copeptin and accounting for about 20% of the primordial precursor has been isolated and sequenced [3].

Transient occurrence of vasotocin ([Ile^3]vasopressin), usually regarded as a non-mammalian neurohypophysial hormone [4], in sheep, seal and human fetuses, has been claimed on the basis of chromatographic, pharmacological and immunological evidence [5–7]. Biochemical research, however, carried out with 7-month- and 3-month-old bovine fetuses, on the one hand, failed to detect vasotocin and, on the other, allowed chemical characterization of oxytocin, vasopressin, MSEL- and VLDV-neurophysins and copeptin, all of which are identical to the corresponding adult polypeptides. These results showed that the same genes are expressed in fetus and adult and that protein precursors are processed in the same way [8–10]. Transient production of vasotocin in mammals would need preservation of an additional 'reptilian' gene expressed only during the early stage of the development and silent in neonate and adult. Identification of vasotocin in the fetus would provide a support to the so-called 'recapitulation law' according to which ontogeny would recapitulate phylogeny. In this respect we have now examined bovine fetus at a very early stage, namely the 1-month-old fetus, and compared the hormones with those of later fetuses and adults.

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2. MATERIALS AND METHODS

2.1. *Pituitary glands*

Nine pituitaries from 1-month-old bovine fetuses have been desiccated in pure and cold acetone and dissected into anterior and posterior lobes. The average weight of the dried posterior pituitary was 1.6 mg. 14.5 mg of powdered tissue have been used for two experiments (7 and 6.5 mg, respectively). Extraction was carried out with hot 0.25% acetic acid (5 min, 100°C). The powder titrated at 0.10–0.13 U/mg uterotonic activity and 0.62–0.79 U/mg pressor activity.

2.2. *Ion-exchange chromatography*

First ion-exchange chromatography was carried out on Amberlite IRC-50 under conditions separating oxytocin, vasotocin and arginine vasopressin [11]. 7 mg of powder were extracted with 5 ml of 0.25% acetic acid and the centrifuged solution passed through a column (0.3 × 21 cm) equilibrated with 0.1 M ammonium acetate, pH 5.0. The hormones were adsorbed at the top and eluted with a pH and ionic strength gradient made first with 0.3 M acetate, pH 7.7, then with 0.6 M acetate, pH 7.7 (mixing flask, 10 ml). The flow rate was 2 ml/h. 0.5-ml fractions were collected and bioassays carried out.

2.3. *High-pressure reverse-phase liquid chromatography (HPLC)*

6.5 mg of powder were extracted with 1.4 ml of 0.25% acetic acid and, after centrifugation and washing twice with 0.2 ml, the pooled solution (1.6 ml) was used for HPLC. A Nucleosil C-18 column (0.46 × 21 cm, particle size 5 μm), was employed with a Waters model 204 liquid chromatograph equipped with a model 680 automated gradient controller, a model 441 fixed wavelength absorbance detector (214 and 280 nm) and a model 730 data module. After equilibration with a solvent containing 15% acetonitrile/85% of 0.01 M sodium acetate, pH 5.0, a linear gradient was applied with a solvent containing 25% acetonitrile/75% of 0.01 M sodium acetate, pH 5.0, for 30 min at room temperature, followed by 5 min with the second solvent, the flow rate being 2 ml/min.

3. RESULTS AND DISCUSSION

3.1. *Ion-exchange chromatographic identification*

The acetic acid extract of posterior pituitary powder containing 1.2 U oxytocic activity and 5.6 U pressor activity has been chromatographed on Amberlite IRC-50 under conditions described and hormones detected by bioassays. Vasotocin is pharmacologically easy to distinguish from oxytocin and vasopressin because it displays both uterotonic (120 U/μmol) and pressor (240 U/μmol) activities. A control experiment with authentic hormones, oxytocin (5 U), vasotocin (0.1 U) and arginine vasopressin (5 U), showed that oxytocin was recovered in tubes 90–110 with a yield of 80%, vasotocin in tubes 160–200 with a yield of 70% and arginine vasopressin in tubes 222–270 with a yield of 74%. In the case of 1-month-old fetus, oxytocin was pharmacologically identified in tubes 90–110 (yield in activity, 70%) and arginine vasopressin in tubes 222–270 (yield in activity, 86%). At the vasotocin position (tubes 160–200), less than 2 mU oxytocic activity with magnesium and less than 6 mU pressor activity were detected. In other words, vasotocin, if present, would be 1% less than that of arginine vasopressin.

3.2. *High-pressure liquid chromatography identification*

The acetic acid solution injected contained 4.5 U pressor activity and 0.77 U oxytocic activity. Two peptides have been pharmacologically detected, one displaying pressor activity and having a retention time of 20.70 min (20.8 min for authentic arginine vasopressin), the other possessing oxytocic activity and having a retention time of 28.20 min (28.2 min for authentic oxytocin) (fig.1). The yields were 87% and 55%, respectively. At the retention time of synthetic vasotocin (13.15 min), and in fractions 14–29, less than 2.8 mU oxytocic activity with magnesium was detected. It can be calculated that less than

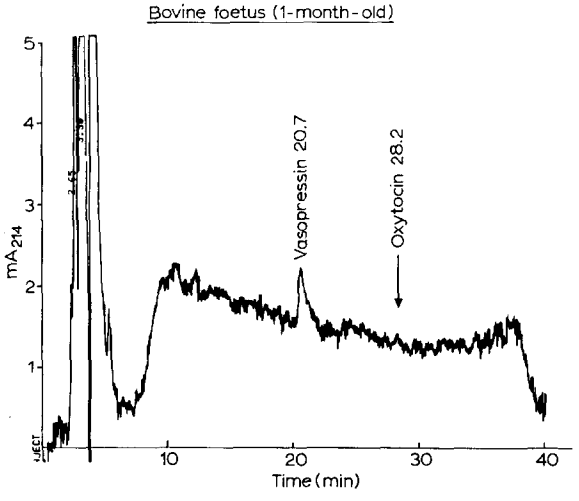

Fig.1. High-pressure reverse-phase partition chromatography of an acetic acid extract from a 1-month-old bovine posterior pituitary. Neurohypophysial hormones have been located by their pharmacological activities (pressor activity for vasopressin, uterotonic activity for oxytocin) and identified by comparison with synthetic peptides. Vasotocin would have been located at 13.15 min.

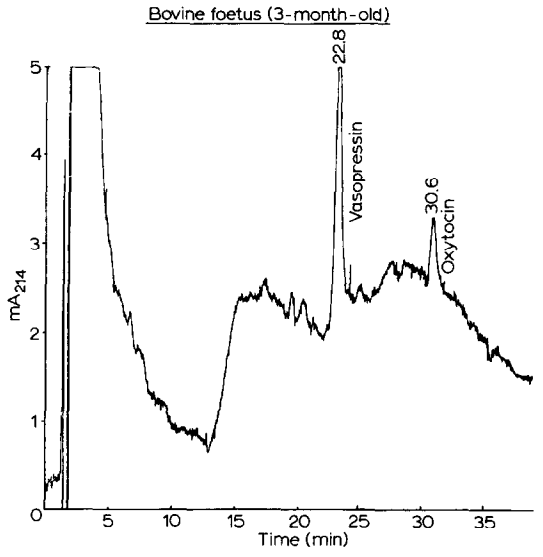

Fig.2. High-pressure reverse-phase partition chromatography of an acetic acid extract from a 3-month-old bovine pituitary. Neurohypophysial hormones have been identified by their retention times and their amino acid sequences.

10 pmol vasotocin corresponding to less than 0.07% arginine vasopressin could be present.

When the same experiment is carried out with posterior pituitaries from 3-month-old bovine fetuses, the same neurohypophysial hormones, vasopressin and oxytocin, can be identified both by retention times in HPLC (fig.2) and microsequencing [10]. Again less than a molar proportion of 0.2% vasotocin compared to arginine vasopressin could be present at the vasotocin position on the chromatogram.

3.3. *Relative expression of oxytocin and vasopressin genes during the development*

The molar ratio of arginine vasopressin to oxytocin, deduced from the biological activities of the powders and the specific activities of the hormones is about 6 in the 1-month-old fetus and about 4 in the 3-month-old fetus. Apparently, the oxytocin gene is expressed much less than vasopressin gene in the very early fetus, probably because the antidiuretic function of the kidney is already working whereas the reproduction activities in which oxytocin is involved (parturition and milk-ejecting action) are not. In several adult mammals such as ox, sheep, pig and rat, the molar ratio of the two hormones is about 1 in the normal animal but it increases in the lactating female due to a large oxytocin secretion [12].

Neurophysins of the 1-month-old fetuses have not been characterized because of the small amount of material but in the 3-month-old and the 7-month-old fetuses, they have been shown to be identical to those of the adult ox [8,10]. We can therefore say that the same genes [13] are expressed during fetal and adult lives and that there is no evidence for an additional vasotocin gene expressed only in the fetus. Vasotocin precursor has been identified in amphibians either directly in the frog *Rana esculenta* [14] or deduced from cDNA in the toad *Bufo japonicus* [15]. The organization in domains appears to be similar to that of the vasopressin precursor and the vasotocin gene probably resembles the vasopressin gene [13,16,17].

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