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学 位 論 文 題 名

Immunocytochemical Analysis of Pituitary Cells in Chum Salmon (*Oncorhynchus keta*)

（シロザケ下垂体細胞の免疫細胞化学的解析）

学位論文内容の要旨

After hatch out in freshwater (FW), salmonid alevins spend in FW up to juvenile stage and then migrate towards the ocean (SW). Attaining maturity in SW, they re-enter their natal river for spawning. The life cycle of many salmonid species thus undergo various physiological challenges. Most of these challenges underlying the migration in salmonids are regulated by pituitary hormones. Therefore, the present study was carried out to examine the cytological features of pituitary cells in chum salmon.

Chapter 1: High population density of juvenile chum salmon decreased the number and sizes of pituitary growth hormone cells.

Chum salmon juveniles held in high population density were apparently smaller than those in medium and low population density. The effects of high population density on pituitary growth hormone (GH) cells were examined in juvenile chum salmon using immunocytochemical and *in situ* hybridization techniques. The ratio of GH-immunoreactive (ir) area to whole pituitary was almost constant in all of the high, medium and low population density groups, although the number and sizes of GH-ir cells were decreased in the high population density group. Image analysis of GH-ir cells indicated the presence of a population of heterogenous cells, in which medium or rather strongly stained smaller cells and weakly stained larger cells as extremes. The medium or rather strongly stained smaller cells were predominated in the high population density group, while weakly stained larger cells in the low population density group. *In situ* hybridization study showed somewhat different distributions and intensities of hybridization signals for mRNAs encoding GH I and II precursors. The area showing signals for GH II mRNA in the high population density fish was significantly smaller than those in the medium and low population density

fish. In contrast, the sizes of areas showing signals for GH I mRNA did not differ among the groups, although the intensity was slightly higher in the high population density fish. These results indicated that high population density decreased the number of weakly immunoreactive larger GH cells, and also suppressed expression of the gene encoding GH II precursor, which may result in retarded somatic growth.

Chapter 2: Immunocytochemical analysis of pituitary cells in pre-spawning chum salmon.

To examine the cytological features of different pituitary cells, pituitary sections were immunostained using antisera against homologous hormones in pre-spawning chum salmon (*Oncorhynchus keta*) caught from the Ishikari Bay (SW) and Chitose river (FW) in October, 1996. Immunoreactivity and cell sizes of growth hormone (GH), prolactin (PRL), somatolactin (SL) and gonadotropin (GTH I and II) cells were measured by a computer-aided image analyzer. Frequency distributions of cell populations were analyzed using a Gauss model. GH-immunoreactive (ir) cells had significantly stronger immunoreactivity with enlarged cell sizes in freshwater (FW) fish than in seawater (SW) ones of both sexes. PRL-ir cells also had significantly stronger immunoreactivity with enlarged cell sizes in FW fish than in SW animals of both sexes. Frequency distributions revealed that almost all PRL-ir cells were strongly immunoreactive in FW fish of both sexes. Immunoreactivity of SL was significantly stronger with enlarged cell sizes in FW fish than in SW ones of both males and females. In addition, greater numbers of strongly stained SL cells were seen in FW animals than in SW ones. These results indicated that PRL and SL cells were stimulated in FW animals regardless of sexes for osmoregulation as well as final maturation. GTH I-ir cells had significantly stronger immunoreactivity with reduced cell sizes in FW fish of both sexes. Moreover, almost all GTH I cells were strongly stained in FW animals compared with SW fish. Conversely, GTH II-ir cells had significantly stronger immunoreactivity with enlarged cell sizes in freshwater (FW) ones of both sexes. Besides, greater numbers of GTH II cells were strongly stained in both FW males and females. These results suggest that GTH II cells were activated with much higher translational activity than secretory activity, while GTH I cells were less potent during spawning migration in salmonids.

The present study concluded that, pituitary GH cells in juvenile salmonid were specifically affected by high population density that reduced secretory activity of GH cells and suppressed the total expression of GH II gene, resulted in retarded somatic growth. In pre-spawning salmonid, PRL and SL cells were stimulated in FW animals than SW ones for

osmoregulation as well as final maturation. However, GTH II cells were activated with much higher translational activity than secretory activity, while GTH I cells were less potent during spawning migration in salmonids.

学位論文審査の要旨

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（シロザケ下垂体細胞の免疫細胞化学的解析）

多くのサケ科魚類は淡水中で産まれ，稚魚期に川を下って海洋を回遊する．やがて成熟すると母川に帰ってきて産卵する．このような回遊という本能的な行動には，多くの生理現象がともなう．それらの生理現象は神経系と内分泌系によって制御されているが，その中でも内分泌系の中枢とされている下垂体の役割は大きいと思われる．したがって，サケ科魚類のライフサイクルにともなう下垂体ホルモン分泌細胞の細胞学的なパラメーターの変動を明らかにすることは，回遊の機構を理解するために必要な，重要な基礎的知見を得ることにつながる．

申請者は，まず，シロザケ (*Oncorhynchus keta*) の稚魚を用いて，成長期の飼育密度と個体の成長との関係および下垂体の成長ホルモン (GH) 産生細胞の細胞学的なパラメーターとの関係を解析した．GH 細胞の細胞学的なパラメーターは，ホモロガスな GH 抗体を用いる免疫細胞化学および切片上ハイブリダイゼーションの後に，画像解析を行うことで定量化している．高密度下で飼育されたシロザケ稚魚は低密度下で飼育された稚魚に較べて体長・体重が少なく明らかに小型であった．低密度下で飼育された稚魚に較べて高密度下で飼育された稚魚では，下垂体そのものが小さく GH 細胞の数が少なかった．画像解析の結果から，下垂体の GH 細胞に中型から小型で免疫染色性の高い細胞とやや大型で免疫染色性の低い細胞が存在すること，高密度飼育によって大型で免疫染色性の低い GH 細胞の比率が大きく減少することが明らかになった．一方，切片上ハイブリダイゼーションの結果は，高密度飼育により GH II 遺伝子（四倍体のサケ科魚類には GH I と II，２種類の遺伝子がある）の発現が抑えられたことを示した．細胞の分布パターンから見ると，高密度飼育により大型で免疫染色性の低い GH II 細胞のホルモン合成活動が抑えられたと考えられる．そして，それが成長速度の低下につながったのであろうと推論している．

次いで，母川回帰時のシロザケを用いて，最終成熟期の個体における下垂体のホルモン産生細胞の様相を免疫細胞化学的に染色し，細胞の大きさと染色性を解析した．動物は石狩湾に面した厚田および千歳ふ化場で入手した．解析したホルモン産生細胞は，成長ホルモン (GH) 細胞，プロラクチン (PRL) 細胞，ソマトラクチン (SL) 細胞，生殖腺刺激ホルモン (GTH-I および-II) 細胞で，母川への遡上と最終成熟にともなって，PRL 細胞，SL 細胞および GTH-II 細胞におけるホルモン合成活動が，その分泌活動を上回って高まっていることが示された．

以上に述べた一連の研究成果は，成長期と最終成熟期というサケのライフサイクルの中でも重要な

時期に，下垂体ホルモン分泌細胞の細胞学的な様相がどのように変化するかを明らかにしたもので，サケ科魚類のライフサイクルの重要な相における生理機能の内分泌学的な調節機構の理解に大きく貢献するものである．

よって，申請者は，北海道大学博士（理学）の学位を授与される資格を有するものと認める．