## Parasitic forms of a myxosporean in the kidney of *Lampetra japonica* : An ultrastructural study

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No information have been available on myxosporea in cyclostomata. The present study was originally planned to investigate "macrophagic cells" in the kidney of arctic lamprey *Lampetra japonica.* Subsequent ultrastructural study revealed that the "macrophagic cells" were unidentified myxosporean trophozoites parasitized at high prevalences in the fish kidney. The trophozoites (pseudoplasmodia with/without sporoblasts) existed predominantly in the lumen of proximal urinary tubules, but were rarely found in any other regions of the kidney. Since no mature spores were found, exact identification of species was impossible. Two types of parasitic forms were observed in proximal urinary tubules: one attaching to the epithelial cells of renal tubules, and the other free-floating in the lumen of tubules. Ultrastructurally, the attaching trophozoites developed microvilli-like projections towards the apical surface of epithelial cells and interdigitated with microvilli of the brush border. In contrast, the whole surface of floating trophozoites was smooth without any cell projections. The developed projections of trophozoites may contribute to their firm attachment to the epithelial cells and/or absorption of nutrients via the epithelial cells. Against the myxosporean infection, the lamprey as the host provoked a local immune reaction by disposition of numerous lymphocytes and macrophages into the epithelium of urinary tubules.

## Contribution of dihydropyridine-insensitive mechanisms to $Ca^{2+}$ influx in rat pancreatic $\beta$ cells

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1. The present study was carried out to clarify the role of $Ca^{2+}$ influx through dihydropyridine-insensitive mechanisms in stimulus-secretion coupling in rat pancreatic $\beta$ cells. To characterize the dihydropyridine-insensitive $Ca^{2+}$ influx, intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) was directly monitored by a microfluorometric method using Fura-2 in isolated perifused preparations of rat pancreatic islets. The changes in $[Ca^{2+}]_i$ induced by high concentrations of glucose and potassium ion ($K^+$) were analyzed quantitatively, and influence of $Ca^{2+}$ entry blockers upon $[Ca^{2+}]_i$ changes was examined.

2. Stimulation of pancreatic islets with 20 mM glucose caused a biphasic $[Ca^{2+}]_i$ dynamics: a transient increase followed by a sustained in-

crease on which recurrent $Ca^{2+}$ spikes were superimposed. The second phase of $[Ca^{2+}]_i$ rise was suppressed by 85% with application of 1 $\mu$M nifedipine, a dihydropyridine-derivative blocker of voltage-dependent L-type $Ca^{2+}$ channels.

3. $K^+$ (50 mM) depolarization induced a rapid rise in $[Ca^{2+}]_i$ consisting of a transient increase followed by a plateau phase. The $[Ca^{2+}]_i$ rise on the plateau phase was suppressed by 30% with application of 1 $\mu$M nifedipine and by 80% by the removal of extracellular $Ca^{2+}$.

4. The nifedipine-insensitive $[Ca^{2+}]_i$ rise was suppressed by 85% by the removal of extracellular $Ca^{2+}$.

5. The nifedipine-insensitive $[Ca^{2+}]_i$ rise was reduced by 20% with application of voltage-dependent N-type and P-type $Ca^{2+}$ channels blockers cocktail (1 $\mu$M $\omega$-conotoxin GVIA, 300 nM $\omega$-agatoxin IVA and 1 $\mu$M $\omega$-conotoxin MVIIC), by 40% with addition of 100 $\mu$M $Ni^{2+}$, a selective T-type $Ca^{2+}$ channel blocker, and by 40% with 2 mM $Ni^{2+}$, a nonselective $Ca^{2+}$ entry blocker, respectively.

6. $K^+$ depolarization-induced $[Ca^{2+}]_i$ rise was dependent on extracellular $K^+$ concentrations, and the maximal $[Ca^{2+}]_i$ rise was obtained at 40 mM $K^+$. The inhibitory effect of nifedipine was also related to extracellular $K^+$ concentrations.

7. These results indicate that the $Ca^{2+}$ entry through voltage-dependent L-type $Ca^{2+}$ channels and dihydropyridine-insensitive mechanisms occurs within membrane potential ranges evoked by high concentration of glucose, and the dihydropyridine-insensitive mechanisms involves N-type and P-type $Ca^{2+}$ channels. Additionally, it is suggested that unknown $Ca^{2+}$ entry pathways exist in rat pancreatic $\beta$ cells.

## Studies on the characteristics of energy metabolism in sheep and shiba goats

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In ruminants, blood glucose levels are consistently low due to their unique feature in digestion and absorption strategy. In central nervous system (CNS), glucose serves as the major energy source in non-ruminants. If this is a ubiquitous law, a question arises how the ruminants compensate for the shortage of glucose supply to their central nervous system. To address this question, an attempt was made by using sheep and shiba goats with implanted probes in their 3rd or lateral ventricles. Cerebrospinal fluids (CSF) were collected through the probe. Previous studies were performed under anesthesia and CSF collection was made at either cisterna magna or lumber spine. This can underestimate possible changes of CSF components. Therefore, in the present experiments, the most suitable region for CSF collection was first determined by comparing several parameters (glucose, lactic acid, pyruvic acid, FFA, insulin, and electrolytes) between 3rd or lateral ventricle and cisterna magna or lumber spine. Then the effects of restricted feeding and fasting on daily plasma and CSF parameters were examined.

1. Xylazine-induced elevation of glucose concentration was larger in CSF collected from the lateral ventricle than that from cisterna magna or lumber spine. Thus, the 3rd or lateral ventricle was considered to be the most appropriate region