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**non-ruminant species.**

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### On-line measurement of released catecholamine and ATP : mechanisms of their release and uptake

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Adenosine-5'-triphosphate (ATP) coexists with catecholamine (CA) in the secretory vesicles of sympathetic nerve terminals and adrenal chromaffin cells. We developed an on-line system for continuous measurement of CA and ATP released from cultured porcine adrenal chromaffin cells and PC 12 cells.

CA and ATP were continuously measured with an electrochemical detector and ATP photometer with luciferin-luciferase, respectively. Acetylcholine (ACh, 0.1 mM) or high K<sup>+</sup> (60 mM) was applied to perfused porcine adrenal chromaffin cells and CA and adenine nucleotides in the collected effluent were analysed with HPLC. The most of CA in the effluent was noradrenarine (81%) and the other was adrenaline (19%). The relative amounts of ATP, ADP and AMP in the effluent were 1, 1.3 and 1, respectively. These ratios were almost the same throughout the period of stimulation.

Using the on-line system, CA and ATP released from cultured porcine adrenal chromaffin cells in response to ACh (0.1 mM) or high K<sup>+</sup> (60 mM) were measured. Increases of CA and ATP in perfused effluent were transient in nature regardless of continuous pres-

ence of the secretagogues and their time courses were almost coincident. The molar ratios of CA to ATP (CA/ATP) in the effluent were  $10 \pm 1$  and  $12 \pm 1$  with ACh and high K<sup>+</sup> stimulation, respectively. In response to repetitive high K<sup>+</sup> stimulation (60 mM), CA and ATP were also released into perfused effluent with the same time courses. Although the amounts of released CA and ATP decreased with repetition, their time courses appearing in the effluent were almost the same and the CA/ATP was  $12 \pm 1$ . Ba<sup>2+</sup> (5 mM) produced rapid increases in CA and ATP, and then the secretory responses declined to about 50% of the peak and sustained during the presence of Ba<sup>2+</sup>. Even in this case, the time courses of CA and ATP appearing in the effluent were also almost the same and the CA/ATP was  $12 \pm 2$ .

The HPLC analysis of CA revealed that the most CA released from pheochromocytoma (PC 12) cells in response to stimulus was dopamine (90%). On-line measurement revealed that high K<sup>+</sup> (60 mM), ACh (0.1 mM) and Ba<sup>2+</sup> (5 mM) caused dopamine and ATP release with almost the same time courses. These secretory responses of dopamine and ATP were quite similar to those of cultured

porcine adrenal chromaffin cells. The molar ratios of dopamine to ATP (dopamine/ATP) in the effluent was  $10 \pm 1$ .

PC 12 cells were cultured with NGF-7 S (50 ng/ml), dexamethasone (1  $\mu$ M), reserpine (0.1  $\mu$ M), bafilomycin A 1 (0.1  $\mu$ M) or without drugs (control) for 2 days and the effects of these drugs on relation of stimulus-secretion coupling and on activity of enzymes which degrade released ATP were examined. The treatment with these drugs did not affect the increase of intracellular  $Ca^{2+}$  and release of relative amounts of adenine nucleotides and adenosine in response to high  $K^+$ . In NGF-treated cells, although neurite production was observed at 2 days treatment, high  $K^+$  caused release of dopamine and ATP with similar amount to those in control cells. Dexamethasone doubled the amount of dopamine release induced by high  $K^+$  without changing the amount of ATP release. High  $K^+$  failed to cause dopamine release in reserpine-treated cells but evoked ATP release with similar

time course and amount to those in control cells. Bafilomycin A 1 decreased both high  $K^+$ -induced dopamine and ATP release.

Based on these results, it is revealed that the molar ratios of CA to ATP released from cultured porcine adrenal chromaffin cells and PC 12 cells are almost constant regardless of the kind of secretagogues. These results suggest that releasable vesicles in both cells contain dopamine and ATP at a constant molar ratio. It is also suggested that released ATP in the effluent from cultured porcine adrenal chromaffin cells is constantly degraded regardless of the kind of secretagogues.

The results obtained from on-line measurement of dopamine and ATP from PC 12 cells which was cultured with various drugs, suggest that dopamine and ATP are stored through different pathways. The  $H^+$ -gradient across the vesicular membrane developed by vacuolar ATPase may play an important role in the vesicular uptake of ATP, like dopamine.

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## Studies on experimental asthmatic model using NC/Nga mice

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The NC/Nga mice have been originally studied as a model for human atopic dermatitis, since they show spontaneous dermatitis accompanied with hyperproduction of IgE. This inherent characters of the NC/Nga mice implied the possibility that they also have

propensity for allergic asthma because etiological relationship among allergic diseases is likely to exist. For these reasons, in this study, the author has established an animal model for allergic asthma using the NC/Nga mice, and investigated the characteristics of