on secretion of catecholamines (CA) from the isolated perfused rat adrenal gland and to establish the mechanism of its adrenomedullary secretion.
The perfusion (0.31ml/min) into an adrenal vein of for 90 min resulted in great increases in CA secretions. Tachyphylaxis to releasing effect of CA evoked by CCCP was not observed by repeated perfusion of it. The net increase in adrenal CA secretion evoked by CCCP still remained unaffected in the presence of pirenzepine or chlorisondamine. However, the releasing effects of CA evoked by CCCP were depressed by *pretreament with pirenzepine, chlorisondamine, nicardipine*, TMB-8, and the perfusion of EGTA plus Ca2+-free medium. CA secretory responses induced by Ach, high K+, DMPP, and McN-A-343 were significantly enhanced in the presence of CCCP (3?0-5 M).
Taken together, these experimental results indicate that CCCP causes the rat adrenomedullary CA secretion in a calcium-dependent fashion, suggesting strongly that this facilitatory effects of CCCP may be mediated by both stimulation of the Ca2+ influx and Ca2+ release from cytoplasmic Ca2+ store.

[PA1-10] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

High Throughput Fluorometric Assay for Cathepsin S inhibitors

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Lysosomal cysteine proteases are involved not only in protein metabolism but also in tissue remodeling, hormone activation and antigen presentation. Among the known cysteine proteases, cathepsin S exists exclusively as a single-chain proteinase. It is also characterized uniquely by its high stability at neutral pH and bell-shaped pH-activity profile. Cathepsin S has received attentions due to its role in the pathogenesis of asthma, Alzheimer's disease, rheumatoid arthritis and other diseases involving tissue destruction. Recently, several evidences demonstrate that selective inhibition of cathepsin S could be a potential strategy for modulating the immune response in autoimmune diseases such as asthma and rheumatoid arthritis.
We established a fluorometric assay with recombinant human enzyme to explore cathepsin S inhibitors from in-house chemical libraries. The assay in the format of 96-well plate is easily adapted for high throughput screening. Therefore, our HTS system can be robustly applicable to the discovery of cathepsin S inhibitor owing to its high sensitivity, precision, accuracy, and stability.

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[PA1-11] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Preclinical studies of CKD-732, an antiangiogenic and antitumor agent.

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We have developed a novel water-soluble fumagillin derivative, CKD-732, and performed preclinical studies as an antiangiogenic antitumor agent. In endothelial cell proliferation assay, CKD-732 was found to show a 72 fold more potent activity compared to fumagillin. In addition, in the Matrigel assay, the hemoglobin content of Matrigel in CKD-732 treatment mice was less than 20% of that in control. Therefore, CKD-732 was found to effectively inhibit a neovessel formation through an angiogenic process. In tumor xenograft models, s.c. injection of CKD-732 induced the growth inhibition of PC-3, CX-1, SKOV-3, LX-1, SNU-16, MDA-MB-231 and Hep3B tumors in a dose dependent manner as much as 64, 74, 69, 69, 68, 70 and 65%, respectively. In animals bearing A375-SM and PC-3 tumors, CKD-732 induced stasis of tumor growth and displayed ILS of >200%. To evaluate the pharmacokinetic property of CKD-732, ADME studies were performed in vitro and in vivo. CKD-732 and 14 metabolites were found from the in vitro samples, and a major metabolite(M11) was identified as a N-oxide form. CKD-732 and M11 exhibited similar plasma kinetic profiles with linear pharmacokinetics, which were detected at 6~8 hrs after an i.v. administration in rat and dog. Therefore, CKD-732 was shown to be relatively stable and to have a long half-life in plasma. These results suggest that the strong antiangiogenic antitumor activity and the improved metabolic