

The thermoregulatory center located in the hypothalamus maintains physiological temperature, while fever depends on the production of exogenous pyrogens, which induce biochemical modifications in the hypothalamus. Exogenous pyrogens can induce the release of endogenous pyrogens or cytokines (IL-1 $\beta$ , IL-6 and TNF- $\alpha$ ). This in turn, leads to the production of PGE. Afterwards, PGE provokes stimulation of the neurons localized within the hypothalamus, thereby triggering a reaction which culminates in the generation of fever. However, the precise mechanism by which endogenous pyrogens or cytokines increase PGE synthesis in hypothalamus is still unclear. Recently, carbon monoxide (CO) was proposed as a novel mediator of the febrile response in the central nervous system. Thus, we studied the interrelationship between heme oxygenase, a CO-producing enzyme, and IL-1 $\beta$ -induced febrile response. IL-1 $\beta$  (1.c.v. inj.) elicited the febrile response and this pyresis was significantly blocked by pretreatment with indomethacin (an inhibitor of COX), but not with ZnPP IX (an inhibitor of heme oxygenase) or ODQ (an inhibitor of soluble guanylate cyclase). IL-1 $\beta$  significantly induced HO-1 in hypothalamus. We couldn't find any consistent evidence that CO is a possible mediator of IL-1 $\beta$ -induced febrile response.

[PA1-40] [ 10/19/2000 (Thr) 10:00 – 11:00 / [Hall B] ]

### **Lipopolysaccharide-induced pyresis is not related to the heme oxygenase induction**

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Peripheral administration of lipopolysaccharide (LPS) induced the release of circulating pyrogenic cytokines, and these cytokines elicit febrile response. Since the structural impermeability of cerebral capillaries and the low efficiency of any transport system for the cytokines across the capillary wall, it was proposed that circulating pyrogenic cytokines have their major effect on the rich vascular network close to the cluster of neurons in preoptic/anterior hypothalamus (ie., organum vasculosum laminae terminalis [OVLT]). However, the precise mechanism by which blood-borne cytokines increase PGE synthesis in hypothalamus is still unclear. In our previous study, i.c.v. injected carbon monoxide (CO) elicited pyresis and this response completely was blocked by indomethacin. Also, CO was proposed as a possible mediator of febrile response in hypothalamus. CO can be produced from heme oxygenase (HO)-catalyzed metabolism of heme. Thus, we studied the relationship of heme oxygenase to LPS-induced febrile response. LPS-induced pyresis was blocked by indomethacin, but not by ZnPP IX (an inhibitor of HO) or ODQ (an inhibitor of guanylate cyclase). LPS (i.p. inj.) did not induce HO-1 in hypothalamus. These results suggest that CO is not involved in LPS-induced pyresis.

[PA1-41] [ 10/19/2000 (Thr) 10:00 – 11:00 / [Hall B] ]

### **The role of G protein in muscarinic receptor-mediated $\alpha$ APPs release in SH-SY5Y cells**

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The amyloid precursor protein (APP) can be processed via several alternative processing pathways. Alpha-secretase processing by cleavage within the amyloid beta-peptide domain of APP is highly regulated by several external and internal signals including G protein-coupled receptors, protein kinase C and phospholipase A2. Stimulation of m1 and m3 muscarinic acetylcholine receptors (mAChR), which are coupled to phosphoinositide hydrolysis and protein kinase C activation, has been shown to increase the release of soluble amyloid precursor protein ( $\alpha$ APPs). There have been several reports indicating that Gq protein-coupled receptors including mAChRs (m1, m3), metabotropic glutamate receptors, and bradykinin receptors, regulate  $\alpha$ APPs secretion. However, there are no direct evidence for the exact roles of G proteins. In the present study, to examine the regulation of Gq protein-linked muscarinic receptor-mediated  $\alpha$ APPs release, we transiently transfected the different G $\alpha$  carboxyl-terminal peptide (G $\alpha$ q, G $\alpha$ i), which have shown a novel dominant-negative strategy (Gilchrist et al.), in SH-SY5Y cells expressing abundant m3 muscarinic receptors endogenously. In wide type cells, increase in  $\alpha$ APPs released by normal metabolism of APP was detected in control medium in a time-dependent manner, and the  $\alpha$ APPs release was stimulated by carbachol, a muscarinic agonist, and phorbol 12-myristate 13-acetate (PMA), a PKC activator. The carbachol-induced increase in  $\alpha$ APPs release was blocked by EGTA, a Ca<sup>2+</sup> chelator, indicating a Ca<sup>2+</sup>-dependent mechanism. On the other hand, PMA-induced  $\alpha$ APPs releases was Ca<sup>2+</sup>-independent. Furthermore, to examine the regulation of  $\alpha$ APPs secretion by upstream cellular signals, dominant-negative G $\alpha$  carboxy-terminal peptide-expressing SH-SY5Y cells were examined, and the results are discussed.

[PA1-42] [ 10/19/2000 (Thr) 10:00 – 11:00 / [Hall B] ]

**The role of intracellular Ca<sup>2+</sup> increase and prooxidant production in the expression of ferritin light chain by sulfur amino acid deprivation in hepa1c1c7 cells**

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Sulfur amino acid deprivation (SAAD) induces oxidative stress through depletion of glutathione content. Ferritin synthesis increases in response to oxidative stress conferring resistance to subsequent insults. However, the molecular mechanisms for the expression of the ferritin gene by oxidative stress have not been studied yet. In the present study, change in intracellular calcium content was determined as part of the complete studies on the expression of ferritin light chain (FLC) gene by SAAD in hepa1c1c7 a murine hepatoma cell line. Confocal microscopy showed that intracellular calcium level was 1.5-fold increased after SAAD up to 80 sec, which extended for the next 200–300 sec, followed by returning to control level. The elevation of calcium by SAAD was prevented by GSH, methionine, cystine or cysteine, indicating that change in the redox-state might control the cellular calcium level. Furthermore, either verapamil or thapsigargin was active in inhibiting the increase in cellular calcium by SAAD, raising the notion that the calcium increase by SAAD might result from the influx of calcium via Ca<sup>2+</sup> channel as well as the release from endoplasmic reticulum. SAAD increased the oxidation of dichlorofluorescein. Treatment of cells with verapamil or deferoxamine, or deficiency of extracellular calcium prevented prooxidant production by SAAD. Hence, elevation of intracellular calcium by SAAD was responsible for the oxidative stress. Northern blot analysis revealed that SAAD increased the mRNA level of FLC, which was inhibited by either EGTA or deferoxamine. Taken together, these results provided evidence that increases in intracellular calcium and oxidative stress by SAAD might lead to the enhanced expression of FLC mRNA in Hepa1c1c7 cells.

[PA1-43] [ 10/19/2000 (Thr) 10:00 – 11:00 / [Hall B] ]

**The changes of catecholamines and indolamines of rat brains by extremely low**