326 DECREASE IN SPINAL A_1-ADENOSINE RECEPTORS AFTER CHRONIC MORPHINE TREATMENT IN RATS

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Recent studies suggest that the release of adenosine and norepinephrine in the spinal cord may be significant components of morphine antinociceptive action. Since we are interested in the chronic effects of morphine, we would like to know whether spinal adenosine receptor or α_2-adrenergic receptor is down-regulated after chronic morphine treatment. Therefore, in the present studies, rats were rendered tolerant to either i.p. or i.c.v. morphine, then spinal A_1-adenosine or α_2-adrenergic receptor binding activities were measured by using [3H]cyclohexyladenosine (CHA) for A_1-adenosine binding and [3H] clonidine for α_2-adrenergic binding. We found there was a significant decrease in spinal A_1-adenosine binding ($p<0.05$) after chronic morphine treatment (both i.p. and i.c.v. administration). However, there was no significant change in spinal α_2-adrenergic receptor binding activities. It implicates that down regulation of spinal A_1-adenosine receptor may play a role in morphine tolerance.

327 THE EFFECTS OF CAPSAICIN AND CAPSAICIN DERIVATIVES ON ISOLATED RAT VAGAL SENSORY NEURONS

Y.-C. Yang *, I.-J. Chen. Department of Pharmacology, Kaohsiung Medical College, Kaohsiung, Taiwan, R.O.C.

The effects of capsaicin and its 4-O-ether linked derivatives on vagal sensory neurons in rat have been investigated in vitro using extracellular recording of compound spike potentials from the whole-nerve by Peak Height Detector Amplifier (PHDA). Capsaicin produced a dose-dependent depolarization in whole-nerve, which shifed the DC level of PHDA upwards, with concurrent changes in compound spike amplitude. When doses were repeated at short intervals, acute tachyphylaxis was evident.

Capsaicin derivatives changed the whole-nerve polarization, but revealed different effects in compound spike potentials.

328 ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISM IN CHINESE DIHYDROPTERIDINE REDUCTASE GENE LOCUS

Y.M. Hong*[1], S.J.Wu[2], T.T. Liu[3], and K.J. Hsiao[1,2,3]. Institutes of Genetics[1] and Biochemistry[3], National Yang-Ming Medical College; Department of Medical Research[2], Veterans General Hospital, Taipei, Taiwan, R.O.C.

Dihydropteridine reductase (DHPR; E.C. 1.6.99.7) catalyses the reduction of quinonoid dihydropteridine to 5,6,7,8-tetrahydrobiopterin (BH_4). BH_4 is the essential coenzyme for three aromatic amino acid hydroxylases which have important biological and physiological functions in higher eukaryotes. Genetic deficiency of DHPR causes phenylketonuria (PKU) associated with progressive neurological disorder. In previous studies, three Chinese PKU patients (two families) with DHPR deficiency were found by us. In order to realize the restriction fragment length polymorphism (RFLP) of DHPR gene locus in Chinese population, human DHPR cDNA was used as probe to analyze twenty-five unrelated and unaffected Chinese DNA samples. Two *Ava*II alleles (9.0/7.0, 5.7/4.3) and two *Nco*I alleles (10/7.8+2.2, 6.6/5.9+0.7/5.9+3.5) were observed. The RFLP frequencies were estimated to be 0.6/0.4, 0.23/0.77, 0.42/0.58 and 0.2/0.78/0.02, respectively. Approximately 64% of Chinese are heterozygous for at least one of the RFLPs. There are no significant difference in the RFLP heterozygosity between Chinese, Caucasians, and Japanese. Both of the two Chinese families with DHPR deficiency have informative markers with these RFLPs, which could be applied to heterozygotes detection and to prenatal diagnosis of DHPR deficiency for genetic counseling.

329 DETERMINATION OF DIHYDROPTERIDINE REDUCTASE (DHPR) ACTIVITY IN AMNIOTIC FLUID CELLS AND ITS APPLICATION FOR PRENATAL DIAGNOSIS OF DHPR DEFICIENCY

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Phenylketonuria(PKU) is an inherited metabolic disease. There are two different types of genetic defect in PKU, namely classical and tetrahydrobiopterin (BH_4) deficient ones. The PKU due to BH_4 deficiency may be caused by defects in dihydropteridine reductase (DHPR), or in BH_4 synthesis. Prenatal diagnosis of BH_4 synthesis deficiency has been achavied by pterins analysis of amniotic fluid. However, for DHPR deficiency, it is not sufficient enough to diagnose prenatally based on pterins analysis of amniotic fluid along. The determination of DHPR activity in amniotic fluid cells was studied for prenatal diagnosis of DHPR deficiency.

The DHPR activity in amniotic fluid cells was determined at 37℃, pH=7.2 by using 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine ($DMPH_4$) as the substrate. The reaction was measured by continuous monitoring of the decrease of NADH at 340nm. The Km of the enzyme for $DMPH_4$ was determined to be 7.0μM. The within-run C.V. of this method was 3.4%. The Chinese reference range of DHPR activity in amniotic fluid cells (n=20) was estimated to be 21.7-71.4 nmol/min/mg protein. The data indicated that DHPR activity is present in normal amniotic fluid cells and may offer the possibility of prenatal diagnosis.

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