Neuroscience Letters (70)

http://www.sciencedirect.com/science/article/B6T0G-43G419D-3/2/0f405388277248866e2855f7e49b0918

The insulin sensitive glucose transporter Glut4 is expressed in neurons of the brain among which those of hypothalamic nuclei. It has been proposed that this transporter might be involved in the hypothalamic glucose-insulin sensing mechanism and thus in the nervous regulation of metabolism. In order to get further insights into its putative role, Glut4 expression was analyzed by quantitative competitive reverse transcription-polymerase chain reaction, in hypothalamic nuclei of hyperglycemic-hyperinsulinemic (HG-HI) rats, a model characterized by alteration of the autonomic nervous system activity. Glut4 mRNA content was decreased in the lateral hypothalamic area (33%) and arcuate nucleus (27%) but significantly only in the former. It was unchanged in other structures. These results are in favor of an alteration of Glut4 expression by short-term hyperglycemia and hyperinsulinemia that, in turn, could affect autonomic nervous system activity.

http://www.sciencedirect.com/science/article/B6T0G-4D99X62-2/2/c95e92003474298f189e2017683c74a6

Mutations in APP are associated with familial early-onset Alzheimer disease (FAD). Examination of the genomic sequence in one patient with FAD revealed a change located in the axon 17 of the APP gene at position 275329G>A (GenBank accession number: D87675; GI: 2429080); cDNA sequence 2137G>A (GenBank accession number: X06989; GI: 28720). This corresponds to the mutation A713T in APP. AD stage VI of neurofibrillary degeneration and stage C of A[beta]-amyloid burden was found at the post-mortem neuropathological examination. Previous studies have suggested that the mutation A713T in APP is a silent mutation or polymorphism. However, we have not found this change in APP in a control population analyzed by the amplification-refractory mutation system (ARMS). It is concluded that A713T in APP is implicated in the pathogenesis of AD. Since the immunohistochemical study indicates that A713T mutation is not likely to relate with A[beta]-amyloid processing, the causative role of this rare mutation remains to be warranted.

Previously, we have reported the cloning and characterization of the 5'-flanking region of the human dopamine D5 receptor encoding gene (D5) and that the major transactivation domain was 119-182 by upstream of the transcriptional start site [Beischlag, T V. et al., Biochemistry, 34 (1995) 5960-5970]. Within this region existed a small dinucleotide repeat termed (TC)13. In this report, we describe the screening of genomic DNAs from 18 unrelated individuals by single-strand conformation polymorphism (SSCP) analysis. SSCP analysis revealed the existence of two additional alleles, termed (TC)12 and (TC)14. Neither form significantly altered D5 promoter-mediated luciferase activity when compared to that of the wild-type control, suggesting that small differences in the number of dinucleotide repeats are not likely of any functional consequence for D5 transactivation.


The molecular mechanisms involved in recovery of function of the central nervous system (CNS) after injury to the brain are incompletely understood. Here the expression of ephrine (Eph) kinases following traumatic brain injury (subdural haematoma) was analysed in order to find out whether these developmentally regulated genes may be involved in tissue remodelling after brain damage. mRNA was isolated from ipsilateral cortices 7, 18, and 28 days after surgery and semiquantitative reverse transcription-polymerase chain reaction was performed. Most Eph kinases did not show significant regulation at gene expression level during the time course of recovery from acute brain injury but there is some evidence that mRNA of EphB1 might be slightly upregulated.


On the basis of the recent cloning of the [beta]-secretase, the beta-site amyloid precursor protein (APP)-cleaving enzyme (BACE), (Science, 286 (1999) 735), digoxigenin-labelled riboprobes were generated to localize the cellular expression pattern of BACE mRNA in brain sections of transgenic Tg2576 mice, overexpressing the Swedish mutation of the APP695 isoform. Non-radioactive in situ hybridization in combination with immunohistochemistry to identify the cell types and [beta]-amyloid deposits revealed strong BACE mRNA hybridization signals in neurons of the cerebral cortex, hippocampal formation, thalamus and cholinergic basal forebrain nuclei, while astrocytes did not display any labeling. Neurons surrounding [beta]-amyloid deposits did not demonstrate altered expression level of BACE mRNA as compared to neurons in cortical areas that are free of [beta]-amyloid deposits, and the regional expression pattern of BACE mRNA did not correlate with the distribution of [beta]-amyloid deposits. These data suggest that high level of expression of BACE mRNA is not necessarily related to enhanced deposition of [beta]-amyloid plaques. To elucidate those factors that contribute to [beta]-amyloid plaque deposition in a...
particular region, the transgenic Tg2576 mouse may represent an appropriate tool.


http://www.sciencedirect.com/science/article/B6T0G-3XDRYT8-9/2/7f9ff944d396a2bb31efc583593e6e10

Reverse transcription-polymerase chain reaction (RT-PCR) was used to characterize the expression of P2X receptor subunits (P2X1-P2X7) in different inner ear tissues. The present study revealed the presence of P2X2, P2X3, P2X4 and P2X7-mRNA in rat organ of Corti, vestibular organ and spiral ganglion at different postnatal developmental stages (PD1-PD16), with slight differences in the onset of expression. Expression of P2X1, P2X5 and P2X6-mRNA was not detectable in the inner ear tissues. In addition, single cell RT-PCR experiments with outer hair cells (OHC) revealed the expression of either the P2X2 or the P2X2-2 splice variant or coexpression of both isoforms in individual cells. Our data suggest that extracellular adenosine-5'-triphosphate (ATP) may play an important role in signal transduction in the inner ear.


http://www.sciencedirect.com/science/article/B6T0G-485RMVR-14T/2/f3b5759369516c4b1c713b997c52732

Interleukin-1 (IL-1) mediates numerous responses on the mouse anterior pituitary cell line AtT-20. We have studied the ligand binding properties of the IL-1 receptors (IL-1R) of the AtT-20 cells and found that they possess 1.5 x 103 receptors/cell with a Kd of 0.15 nM for [125I]IL-1[alpha]. Using oligonucleotide primers, which define a 236 bp region of the cDNA coding for the cytosolic part of the mouse T-cell IL-1R, and cDNA prepared from AtT-20 cells, we obtained by polymerase chain reaction (PCR) a DNA fragment which was shown to possess an identical sequence to that of the corresponding region of the mouse T-cell IL-1R. Thus the AtT-20 pituitary cell line IL-1 receptor appears to be similar to those found on mouse T-cells and fibroblasts.


http://www.sciencedirect.com/science/article/B6T0G-475BFKS-6/2/76b7a7468f02acb06afad5c5a814ae6e

The neuronal nicotinic acetylcholine receptor subunit, [alpha]7, can form homopentameric receptor/ion channel complexes. Potential contributions of its N-terminal region to homomeric interactions were investigated, in comparison with the corresponding region of an analogous heteromeric subunit, [alpha]3. Recombinant chimeras were prepared upon engineering the N-terminal [alpha]7 (M1-V224) or [alpha]3 (M1-S232) sequence into the background of another homomeric mouse 5-hydroxytryptamine3 (5-HT3) receptor. The [alpha]7/5-HT3 chimera, when expressed heterologously in a human epithelial cell line, SH-EP1, robustly expressed [alpha]7-bungarotoxin binding sites as homooligomers while the [alpha]3/5-HT3 did not produce
epibatidine (non-selective ligand) binding sites, and did not interfere the [alpha]7/5-HT3 phenotype, upon co-expression. Yeast two hybrid assays with the N-terminal regions showed positive responses between [alpha]7:[alpha]7, but not between [alpha]7:[alpha]3 and [alpha]3:[alpha]3. Similar assays with the [alpha]7 N-terminal region and its five smaller fragments (G23-N46, D47-N90, V91-N133, S134-M182 and Q183-V224) revealed that the G23-N46 sequence is involved in homomeric interactions. Replacement of the corresponding region of the [alpha]3/5-HT3 chimera with the [alpha]7 G23-N46 sequence conferred a dominant negative role on the chimera, by abolishing the [alpha]7/5-HT3 phenotype. These results support the view that the G23-N46 portion of the [alpha]7 N-terminal region may contribute to receptor homooligomerizations.


In order to develop in vitro models of CNS injury, astrocytes have been mechanically injured in culture to study reactive astrocytosis. However, scratch injury models of pure neuronal cultures have not yet been exploited to study programmed cell death (PCD). For this study, we examined model motor neurons (NSC19 cells) in culture and found time-dependent cell death in proximity (within 2.5 mm) to a physical scratch injury. Injury-induced cell death was apoptotic verified by positively-stained nuclei using both the in situ end-labeling (ISEL) procedure and Hoechst 33342. Unexpectedly, cells proximal to the injury site were not affected by the injury until 3 days later suggesting that adjacent motor neuron loss was dependent on a 'death signal' produced by direct injury to sister neurons. 'Executioners' in apoptosis include free radicals, cell cycle kinases and cysteine proteases (caspases). Extracellular serine proteases, such as thrombin and granzyme B, may activate such intracellular pathways and several inhibitors (serpins), such as CrmA, are effective in blocking apoptosis. Since protease nexin I (PNI), a serpin homologous with CrmA, prevents apoptosis of lumbar motor neurons and is increased after nerve injury, we examined mRNA by RT-PCR for PNI expression. Of interest, although we were unable to find significant levels of PNI message in NSC19 cells, we did detect it in the parent neuroblastoma.


This study examined the influence of a variation in the KLOTHO gene on cognitive ability at age 11 and age 79 in 464 people from the Lothian Birth Cohort 1921 (LBC1921), and at age 11 and age 64 in 451 people in the Aberdeen Birth Cohort 1936 (ABC1936). In the LBC1921, people with the KLOTHO V/V genotype had lower verbal reasoning ability at age 11 and age 79, and lower non-verbal reasoning at age 79, than those with the F/F genotype, or heterozygotes. The effect of the KLOTHO polymorphism on cognition at age 79 was non-significant when adjusted for IQ at age 11. In this sample, KLOTHO V allele status accounts for about 2% of the variance in life-long traits related to verbal and non-verbal reasoning, but not to age-related cognitive change. These results were not replicated in the ABC1936 sample. In a combined analysis of the LBC1921 and the ABC1936 cohorts there was a significant KLOTHO X sex interaction: women with the V/V genotype had lower non-verbal reasoning scores at age 79, after adjustment for cognitive ability at age 11. Variation in the KLOTHO gene is a possible contributor to life-long reasoning differences in humans and/or to the ageing of non-verbal reasoning, especially in women.

http://www.sciencedirect.com/science/article/B6T0G-3S0MJGM-K/2/10e9918dad20125e44beb6b5b66a7dce

Reverse transcriptase polymerase chain reaction (RT-PCR) using primers for the recently cloned human CGRP1 receptor detected mRNA expression of CGRP1 receptors in trigeminal ganglia and cerebral vessels, obtained at autopsy or during neurosurgical tumor resections. An RT-PCR product of the expected size (339 bp) was seen in cerebral arteries, both in the presence and in the absence of endothelium and in trigeminal ganglia. Sequence analysis of the RT-PCR product of the published sequence showed 100% homology with the human CGRP1 receptor. The presence of the CGRP1 receptor mRNA in human trigeminal ganglia and cerebral blood vessels, indicates the occurrence of both prejunctional (trigeminal) and postjunctional location (blood vessels) of the CGRP1 receptor.


http://www.sciencedirect.com/science/article/B6T0G-3VCMTPS-1Y/2/2024509d65296458a89fa6c63aeacc47

Both muscarinic and nicotinic acetylcholine (ACh) receptors are known to be present on the surface of lymphocytes. We have shown that variable amounts of ACh are detectable in the blood of various mammals including humans, and a major portion of blood ACh is localized in circulating mononuclear leukocytes in humans. In order to investigate which types of blood cell are the source of ACh in human blood, expression of mRNA for choline acetyltransferase (ChAT, EC 2.3.1.6), which catalyzes ACh synthesis, was analyzed using human leukemic cell lines as models of lymphocytes and the reverse transcription-polymerase chain reaction (RT-PCR) method. We observed that mRNA for the same ChAT as that in the nervous system is expressed constitutively in all the T-cell lines tested, but not in B-, pre-lymphoma or monocytic cell lines. Furthermore, only T-cell lines showed high ACh-synthesizing activities and intracellular ACh contents. These results suggest that the major portion of ACh in the circulating blood originates from T-lymphocytes.


http://www.sciencedirect.com/science/article/B6T0G-3PV1RMM-10/2/f90855252471efa519a8aaee8c4f8a9a5b

Timed-pregnant Sprague-Dawley rats were killed between gestational day (GD) 8 and 10, and embryos were explanted and separated into developmental stages according to a modified Theiler's system. Total RNA from each stage was isolated and subjected to reverse transcription-polymerase chain reaction (RT-PCR) assays to examine gene expression of catecholamine synthesizing enzymes and three subtypes of [beta] adrenoceptors. Expression of these genes was detected at much earlier stages than previously reported, and each enzyme and receptor subtype showed a different pattern of gene expression. For example, mRNA for tyrosine
hydroxylase, the rate-limiting enzyme for catecholamine synthesis, was detected as early as stage 10a, late GD 8, before the neural crest cells appear (stage 12, mid GD 10). This contradicts the common belief that catecholamines are produced only in the cells of sympathoadrenal lineage which originate from the neural crest cells and the cells of central nervous system. Results from the present study indicate that catecholamine synthesis is not limited to the cells of sympathoadrenal lineage.


http://www.sciencedirect.com/science/article/B6T0G-3YB9Y46-S/2/daf8d69aa9575f1891d7dcbb64b4a188

The trembler mouse suffers from a dominantly inherited mutation of the peripheral myelin protein 22 (PMP22) gene which results in an abnormal myelination of its peripheral nervous system. The recent identification of two different PMP22 mRNA differing in their 5' non-translated region led us to monitor their respective levels of expression in the trembler peripheral nervous system (PNS) during the myelination period. We showed that the steady-state levels of the exon 1A-containing transcript, which is thought to be involved in the myelination process, were greatly reduced in heterozygous and homozygous trembler mice when compared to the normal animals. Such a difference was not observed for the exon 1B-containing transcript. Therefore, our results support the idea that the two alternatively used promoters of the PMP22 gene are under different regulation control, and that the up-regulation of the exon 1A transcript is necessary for the normal myelination of the mouse PNS.


http://www.sciencedirect.com/science/article/B6T0G-42P51GH-F/2/baa6904cfd640c11fb7fee88586129bf

Cerebellar granule neurons can be maintained in culture in a medium containing high serum and depolarising levels of KCl. When serum is removed and the KCl levels lowered from 25 to 5 mM, the cells undergo apoptosis. Apoptosis can be prevented by inhibitors of transcription or translation, suggesting a need for macromolecular synthesis in the apoptotic process. Using quantitative reverse transcription-polymerase chain reaction the levels of mRNA for a range of genes postulated to be important in apoptosis have been examined. Elevated levels of caspase 3, c-Jun, and Fas ligand were found, in addition to a corresponding increase in c-Jun protein and activation of caspase-3. These results suggest that cerebellar granule neurons upregulate components of both death receptor-mediated and the mitochondrial-mediated death pathways.


http://www.sciencedirect.com/science/article/B6T0G-4D9DF7J-1/2/c248abc18176ba6011382cde43c40ee

The glutamateergic dysfunction hypothesis of schizophrenia suggests genes involved in
glutamatergic transmission as candidates for schizophrenia-susceptibility genes. It has recently been reported that some haplotypes in the AMPA receptor subunit GluR4 Gene (GRIA4), which is located on chromosome 11q22, are positively associated with schizophrenia in the Japanese population. In order to assess the role of GRIA4 in schizophrenia, we examined three reported positive SNPs (single nucleotide polymorphisms): rs609239, rs641574 and rs659840 at the GRIA4 locus in schizophrenic cases (n = 372) and controls (n = 392) of the Chinese population. Although we had observed similar allele and genotype frequencies compared with that in the Japanese population, no evidence was found for association with the disease in the analysis of either single nucleotide polymorphisms (all P-values > 0.300) or haplotype relative risk (all P-values > 0.088). Our results suggest that the three SNPs of GRIA4 are unlikely to play a major role in the susceptibility to schizophrenia in the Chinese population.


http://www.sciencedirect.com/science/article/B6T0G-3W788DW-15/2/f18eb33a7368297ae9bc735fffbff85f

The effect of acyclovir treatment on viral burden and the expression of immunologic nitric oxide synthase (iNOS) within brains of 42 HSV-1F infected mice was studied by using a titration PCR assay for HSV-1 DNA and a semiquantitative RT-PCR for iNOS mRNA. iNOS mediated NO-production may possibly be involved in secondary mechanisms of brain injury following virus infection, which may account for treatment failures in human herpes simplex virus encephalitis (HSVE). Following infection, a parallel increase of iNOS mRNA and HSV-1F-DNA occurred with peaks after 7 days that were both significantly lower under acyclovir treatment. Six months post infection viral load had declined, but iNOS mRNA expression in both treated and untreated mice was still enhanced as compared with mock infected controls. This suggests that acyclovir decreases iNOS expression via inhibition of viral replication shortly after infection but fails to influence elevated iNOS within the brain late in the course of experimental HSVE.


http://www.sciencedirect.com/science/article/B6T0G-452WHMW-2/2/85051b0b00a8588e88fa3546db81e3b8f

In Alzheimer's disease (AD), amyloid plaques within the brain are surrounded by activated glial cells (microglia and astrocytes). The mechanisms of glial activation and its effect on disease progression are not fully understood. Growing evidence suggests that beta-amyloid (A[beta]) peptide, a major constituent of the amyloid plaque, is critically involved in the induction of an inflammatory response. The goal of this study was to examine the role of A[beta] in the pathogenesis of local inflammation and neuronal cell death. We found increased mRNA levels of inducible nitric oxide synthase (iNOS) and the arginine regenerating enzyme argininosuccinate synthetase (ASS) within cortices of AD patients suggesting high output NO production. In vitro, synthetic A[beta]1-42 and to a lesser extent A[beta]1-40 induced iNOS and ASS transcription with consecutive NO overproduction in mixed rat neuronal-glial cultures. Furthermore, A[beta]-stimulation lead to an increased release of inflammatory cytokines interleukin (IL)-1[beta], IL-6 and tumor necrosis factor-[alpha]. Again, A[beta]1-42 had a much more pronounced effect as compared to A[beta]1-40. Our data suggest that A[beta]1-42 is a key mediator of glial activation and via the induction of inflammatory mediators may be a critical component of the
neurodegenerative process in AD.


http://www.sciencedirect.com/science/article/B6T0G-3XPDB8W-7/2/fd251f68e4ecb1e1931e971b3fd90db72b

The occurrence and distribution of the muscarinic M2-receptor subtype (M2R) was investigated in rat thoracic dorsal root ganglia (DRG). Messenger RNA for M2R was demonstrated by RT-PCR in total RNA from DRG. Immunoreactivity to M2R-protein was localized to 26% of sensory neurons, the majority of them (85%) belonging to the size class of 25-40 [mu]m in diameter. Double-labeling (immuno)histochemistry revealed that all M2R-immunoreactive neurons bind the lectin, I-B4, whereas they are generally devoid of substance P-immunoreactivity. These data show the presence of M2R on a subpopulation of presumably nociceptive primary afferent neurons, thereby extending previous pharmacological and electrophysiological studies that indicated a role of M2R and/or M4R in inhibition of calcium channel currents in rat sensory neurons (Wanke, E., Bianchi, L., Mantegazza, M., Guatteo, E., Macinelli, E. and Ferroni, A., Muscarinic regulation of Ca2+ currents in rat sensory neurons: channel and receptor types, dose-response relationships and cross-talk pathways. Eur. J. Neurosci., 6 (1994) 381-391).


http://www.sciencedirect.com/science/article/B6T0G-483SX2P-9/2/35a784b583ec9b9cf8b8b1fd883512

3[beta]-hydroxysteroid dehydrogenase (3[beta]-HSD) is an enzyme that converts pregnenolone to progesterone. It has been believed that 3[beta]-HSD is simply a converting enzyme of female steroid hormone. Recently, 3[beta]-HSD expressing cells were identified in the spinal cord. Steroid synthesis in the nervous system may indicate that steroid plays a role in the nervous system. We report here the increased expression of 3[beta]-HSD mRNA in the dorsal root ganglion (DRG) after peripheral nerve injury using reverse transcription-polymerase chain reaction and in situ hybridization histochemistry techniques. We detected only a few 3[beta]-HSD signals in the naive DRG, and found that 3[beta]-HSD mRNA expression increased 3 days after injury and this increase was still observed at 14 days. Our results suggest that progesterone may have a role in the process against neuronal injury or in regeneration in the peripheral nervous system.


http://www.sciencedirect.com/science/article/B6T0G-46SK5FH-4/2/ed048e31ed56eda06cc837a6aa3b4d80

The cloned capsaicin receptor, also known as vanilloid receptor subtype 1 (VR1) receptor, has been demonstrated to be an integral membrane protein with homology to a family of putative store-operated calcium channels. The VR1 receptor is activated not only by capsaicin but also by
noxious heat and protons, and therefore it is suggested as a molecular integrator of chemical and physical stimuli that elicit pain. In the present study, indirect immunofluorescence detected a small number of neurons that are VR1 receptor immunoreactive (ir) (171 versus 1038 or 16% of all neuronal cell bodies) in the human trigeminal ganglion (TG). In addition, RT-PCR confirmed the presence of VR1 mRNA in the human TG. It has been hypothesized that TG neuronal cell bodies are the source of capsaicin-stimulated release of calcitonin gene-related peptide (CGRP), and hence co-localization experiments were performed. Around 10% of the VR1 receptor-ir is expressed on neurons that contain CGRP-ir (ten among 74) in the human TG, indicating that capsaicin may act through the VR1 receptor to modulate the release of CGRP and in turn to modulate pain. We observed that 8% of the VR1 receptor-ir neuronal cell bodies contain substance P-ir and 5% nitric oxide synthase. Capsaicin can release nitric oxide, CGRP and substance P from sensory nerves and contribute to central sensitization.


http://www.sciencedirect.com/science/article/B6T0G-48NXD5P-F/2/1f8e4f7e541f4bb717b65bccc445cb0e

Hypofunction of glutamatergic neurotransmission has been hypothesized to underlie the pathophysiology of bipolar affective disorder, as well as schizophrenia. We examined the role of the N-methyl--aspartate receptor 2A subunit (GRIN2A) gene on 16p13.3, a region thought to be linked to bipolar disorder, (1) because in a prior study we identified a functional and polymorphic (GT)n repeat in the 5' regulatory region of the gene, with longer alleles showing lower transcriptional activity and an over representation in schizophrenia, and (2) because of the suggestion of a genetic overlap between affective disorder and schizophrenia. Family-based association tests detected a nominally significant preferential transmission of longer alleles in a panel of 96 multiplex bipolar pedigrees. These results support the hypothesis that a hypoglutamatergic state is involved in the pathogenesis of bipolar affective disorder.


http://www.sciencedirect.com/science/article/B6T0G-4F662KF-5/2/6c599fb523146d134ce7ee96a74383bb

Dysfunction of the N-methyl-d-aspartate (NMDA) type glutamate receptor has been proposed as a mechanism in the etiology of schizophrenia. Recently, we identified a variable (GT)n repeat in the promoter region of the NMDA NR2A subunit gene (GRIN2A), and showed its association with schizophrenia in a case-control study, together with a correlation between the length of the repeat and severity of chronic outcome. In this study, we extended our analyses, by increasing the number of case-control samples to a total of 672 schizophrenics and 686 controls, and excluded potential sample stratification effects. We confirmed the significant allelic association between the repeat polymorphism and disease (P = 0.011), and as in the previous study, we observed an over-representation of longer alleles in schizophrenia. These results suggest a probable genetic effect for the GRIN2A promoter (GT)n variation on the predisposition to schizophrenia in Japanese cohorts.

http://www.sciencedirect.com/science/article/B6T0G-40BPR2M-F/2/c3e6ffeb12a98d04ab8b28edb8194b39

We have analyzed the size of the expanded poly(CAG) associated with juvenile Huntington disease in the cerebra and the cerebella of five patients. The expanded poly(CAG) was always longer in the cerebrum than in the cerebellum, but the difference in size varied from patient to patient. Except for one patient who possessed an unusually large expansion, very little heterogeneity of size was detected within the cerebrum or within the cerebellum. The larger size of the expanded poly(CAG) in cerebrum must therefore have resulted from a single expansion event that took place early in cerebral development. In both cerebrum and cerebellum, the size of the expanded allele of gray matter was identical to that of white matter. We conclude that most if not all neurons and glia of cerebrum are descended from a common bipotent precursor, which segregated early in neurogenesis from the lineage leading to cerebellar neurons and glia.


http://www.sciencedirect.com/science/article/B6T0G-3W4XHF1-G/2/8a804ab054c32a0209c722c34b9e53825

Using a combination of polymerase chain reaction (PCR), single-strand conformation polymorphism (SSCP) and DNA sequencing techniques, we identified a unique missence mutation (T->C) in exon 3 of the APOE gene which resulted in the substitution of pro-28 for leu-28. We screened 1118 White cases of late-onset (>60 years) Alzheimer's disease (AD) from three independent centers (Pittsburgh=489, Indiana=319, Mayo Clinic Rochester=310) and 1123 controls (607 clinically assessed and 516 individuals randomly ascertained from the general population). Two of the 1123 control subjects had the pro-28 mutation (0.18%). However, this mutation was observed in heterozygous state in 2.66, 2.51 and 1.94% of the AD cases from Pittsburgh, Indiana and Mayo Clinic Rochester, respectively, with an overall frequency of 2.42%. All individuals with this mutation were carriers of the APOE*4 allele and hence the mutation was denoted as APOE*4 Pittsburgh (APOE*4P). Compared with the non-E*4P carriers, the E*4P carriers were associated with an increased risk of AD (odds ratio (OR) 13.2) and this risk remained significant even after adjusting for the known effect of APOE*4 (OR 5.4). The risk associated with the E*4P/E*4 combination was about five times (OR 29.1) the risk attributed to APOE*4 carriers alone (OR 5.7). Our data indicates that the new mutation most likely exists in cis-orientation with APOE*4 and is associated with increased risk of developing AD.


http://www.sciencedirect.com/science/article/B6T0G-3VXJJY5-F/2/35112c05f86a1d27e3ea73dc968bc8ab

Presenilin-1 (PS-1) gene of three Japanese pedigrees with early-onset familial Alzheimer's disease (FAD) disclosed two novel missense mutations resulting in Va196Phe and Ile213Thr, and one mutation resulting in His163Arg. The mean age at onset in a family with His163Arg mutation
was similar to those reported in other families with His163Arg. Our results suggested the existence of a variety of PS-1 mutations, and that early-onset FAD with PS-1 mutations is highly penetrant and is only rarely subject to modulation by genetic or environmental modifying factors.


http://www.sciencedirect.com/science/article/B6T0G-4D3B3V4-4/2/69caf10c4820e608ee6102a01be4e8ca

The purpose of this study was to investigate whether orexin expression in the rat brain was changed during pregnancy. Brain samples were obtained from 5 nonpregnant rats and 10 pregnant rats (5; day 10 of gestation, and 5; day 20 of gestation). Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis was performed to investigate the expression of prepro-orexin mRNA and the housekeeping gene in the rat brain. The signals were quantified by the densitometric analysis. The distribution and expression of orexin-A and orexin-B were determined using immunohistochemistry. The ratio of the prepro-orexin mRNA expressions to the housekeeping gene expression in pregnant rat brain were significantly higher than that in nonpregnant control. There was no significant difference between prepro-orexin mRNA levels of day 10 and day 20 of gestation. Immunohistochemical staining for orexin-A and orexin-B was present in neurons within and around the lateral and posterior hypothalamic areas in both nonpregnant and pregnant rats. These results suggest that increased prepro-orexin mRNA levels at early gestational age in the maternal rat has a role on energy metabolism during pregnancy.


http://www.sciencedirect.com/science/article/B6T0G-46F6ST6-5/2/9a59d1eeec4678f058d9f93c3e45b1210

The family of neurotrophins, encompassing nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5), is important in the regulation of neuronal development and function. We examined the expression of neurotrophin messenger RNAs (mRNAs) in the rat urinary bladder during pre- and postnatal development using competitive reverse transcription-polymerase chain reaction. The mRNA levels showed a biphasic pattern of expression; one peak was at prenatal ages (embryonic day (E)15-E18) and the other peak was at postnatal ages (postnatal day (P)14-P28). NT-4/5, BDNF and NGF mRNA levels were greatest at E15, E16 and E18, respectively. In contrast, NT-3 mRNA levels were significantly highest at P14. These data suggest that neurotrophins are involved in the mechanisms of bladder nerve growth for the prenatal period and reorganization of bladder reflex pathways during the second to the fourth postnatal week.


http://www.sciencedirect.com/science/article/B6T0G-42FS95B-6/2/ff4c014a14a8468aa237e061a5c641a0

Aquaporin-4 (AQP4) is the most abundant water channel in the rat brain. In this study, the
distribution pattern and mRNA expression levels of AQP4 were examined in a severe traumatic brain injury model by immunohistochemistry and reverse transcription-polymerase chain reaction. Oedema formation and blood-brain barrier (BBB) integrity were assessed by wet-dry weight measurements and immunostaining of endogenous IgG respectively. In the oedematous contusional cortex with impaired BBB integrity, negative immunostaining of AQP4 and down-regulation of its mRNA level were identified (P<0.05) at 1 day post-injury, while in other oedematous regions of the injured brain where BBB was intact, there was no significant change in the AQP4 expression level. This heterogeneous pattern of AQP4 responses can be interpreted as follows: focal brain injury (such as a contusion) with impaired BBB resulting in vasogenic oedema is associated with reduction of AQP4 expression, whereas, in cytotoxic oedema, associated with diffuse brain injury with intact BBB, changes in AQP4 expression are not significant. This study provides basic information for investigating new treatments for traumatic brain oedema.


Activation of cytosolic phospholipase A2 (cPLA2) is an early event in brain injury, which leads to the formation and accumulation of bioactive lipids: platelet-activating factor (PAF), free arachidonic acid, and eicosanoids. A cross-talk between secretory PLA2 (sPLA2) and cPLA2 in neural signal transduction has previously been suggested (J Biol Chem 271:32722; 1996). Here we show, using neuronal cell cultures, an up-regulation of cPLA2 expression and an inhibition by the selective cPLA2 inhibitor AACOCF3 after exposure to neurotoxic concentrations of sPLA2-OS2. Pretreatment of neuronal cultures with recombinant PAF acetylhydrolase (rPAF-AH) or the presynaptic PAF receptor antagonist, BN52021, partially blocked neuronal cell death induced by sPLA2-OS2. Furthermore, selective COX-2 inhibitors ameliorated sPLA2-OS2-induced neurotoxicity. We conclude that sPLA2-OS2 activates a neuronal signaling cascade that includes activation of cPLA2, arachidonic acid release, PAF production, and induction of COX-2.


In our previous studies, we found that behavioral sensitization evoked by repeated administration of methamphetamine (METH) was suppressed by the activation of the histaminergic neuron system in the brain. In continuation of these studies, we measured the levels of H1 and H2 receptor mRNAs in the rat striatum by semi-quantitative reverse transcription-polymerase chain reaction. Seven days after the 21 consecutive administrations of METH (4 mg/kg, i.p.), the levels of both H1 and H2 receptor mRNAs in the rat striatum increased significantly. However, 1 and 14 days after the last administration, there were no significant changes in levels of either H1 or H2 receptor mRNA in the rat striatum. These transient increases of H1 and H2 receptor mRNAs may have some relation to chronic METH abuse and its withdrawal.

http://www.sciencedirect.com/science/article/B6T0G-3VCMTTPS-2C/2/7cb2b2eab62d30d90b0f74d586686ce5d

The occurrence and distribution of the preferred receptor for the neuropeptide, substance P (SP), the neurokinin-1 receptor (NK1R) was investigated in the vascular supply of the rat sciatic nerve. Messenger RNA for NK1R was demonstrated by RT-PCR in the epineurial layer where the majority of small arteries and arterioles feeding the endoneurial vasculature are located. Immunoreactivity to NK1R-protein was localized on the smooth muscle cells of these arterial vessels by means of immunofluorescence using a polyclonal NK1R antiserum. This muscular localization of NK1R explains the previously reported [Zochodne, D.W. and Ho, L.T., *J. Physiol.*, 444 (1991) 615-630] moderate vasoconstrictor rather than vasodilator effects of SP in this vascular bed.


http://www.sciencedirect.com/science/article/B6T0G-4D09M7P-1/2/515fa9ee5b79f26fbd7fae5c5113506f

Psychotic patients treated with clozapine often experience persistent daytime sleepiness. This is a frequent side effect of clozapine that may reduce patient compliance. We hypothesized that clozapine might interfere with the circadian rhythms regulated by the biological clock. In 171 patients with major psychosis, we investigated the association between hypersomnolence during clozapine therapy and a CLOCK gene polymorphism (3111 T/C substitution). Forty-six patients showed persistent daytime sleepiness and were classified as "sleepy". "Sleepy" patients were significantly more likely to have a mutated allele compared to both "non sleepy" patients and healthy subjects ($\chi^2 = 20.36$, d.f. = 1, $P = 0.000007$, and $\chi^2 = 13.91$, d.f. = 1, $P = 0.0002$, respectively). We conclude that an interaction between clozapine and the CLOCK gene polymorphism 3111 T/C substitution could explain persistent daytime sleepiness in a significant proportion of patients treated with clozapine.


http://www.sciencedirect.com/science/article/B6T0G-3T11VR4-9/2/84527275253272edc8755a00be75ca79

Secretoneurin is a recently-characterized neuropeptide derived from secretogranin II, a protein belonging to the class of chromogranins. We investigated the phylogeny of this peptide by immunoblotting and gel-filtration high performance liquid chromatography followed by radioimmunoassay of brain extracts of various species including chicken, lizard, frog and fish. In addition the amino acid sequence of secretoneurin from pig, hamster, rabbit, guinea-pig and chicken was established by reverse transcriptase polymerase chain reaction. Secretoneurin is strongly conserved during evolution, it is not only expressed in various mammalian species but found also in the brain of birds, reptiles, amphibians and fish. In all these species a significant or near complete processing of secretogranin II to secretoneurin was observed. These data provide significant evidence for the neuropeptide nature of the novel functional peptide.
The calcitonin receptor-like receptor (CRLR) and the orphan receptor RDC-1 have been proposed to be calcitonin gene-related peptide type 1 (CGRP1) receptors, and receptor activity-modifying proteins (RAMPs) determine the ligand specificity of CRLR. Coexpression of RAMP1 and CRLR resulted in functional CGRP1 receptors; the complex of RAMP2 or RAMP3 and CRLR created functional adrenomedullin receptor. Although high levels of CGRP binding sites in the nucleus accumbens have been reported, little is known about the expression of these novel CGRP receptors. In the present study, we used real-time quantitative RT-PCR to detect and quantitate the relative expression of CGRP, CRLR, RAMP1-3 and RDC-1 in the nucleus accumbens of intact rats and rats with inflammation. Our results demonstrate that CGRP, CRLR, RAMP1 and RAMP2 exist in the nucleus accumbens of intact rats, and that they were significantly upregulated in rats with inflammation. In contrast, no expression was detected for RDC-1 and RAMP3. These findings indicated a functional role for CGRP and its receptors in inflammation and pain modulation.


Serotonin type-3 (5-HT3) receptors are cation permeable membrane receptors which are involved in modulation of calcium entry in neuronal cells. Along with other ion-channels such as the N-methyl-aspartate receptor, it appears to be a target for the actions of ethanol and has been the focus of considerable work in this regard. Since in animals, ethanol exposure results in elevations of corticosteroids in both acute and chronic conditions, we studied the effects of both ethanol and corticosteroid exposure on 5-HT3 gene expression in an in situ pheochromocytoma-12 (PC12) cell model. We found that ethanol exposure alone (80 mM x 4 days) did not significantly alter target gene expression. Corticosterone (CORT) (50, 150, and 300 ng/ml) resulted in significant increases in 5-HT3 expression which were attenuated by mifepristone (50 ng/ml). Ethanol in combination with CORT did not significantly alter the increase in 5-HT3 mRNA seen with CORT alone. We conclude that in PC12 cells, exposure to CORT at physiologically relevant concentrations increases 5-HT3 gene expression.


This study was conducted to determine whether the rat suprachiasmatic nucleus (SCN) is characterized by circadian expression of brain-derived neurotrophic factor (BDNF). In constant darkness, SCN content of both BDNF mRNA and protein oscillated in a circadian fashion. BDNF mRNA and protein levels in the SCN reached peak values during the early subjective day and
during the subjective night, respectively. In contrast, the hippocampus showed no sign of circadian rhythmicity in its expression of BDNF mRNA and protein. Since BDNF enhances synaptic transmission in other brain regions, the coincidence between peak expression of BDNF protein in the SCN and the known interval of circadian pacemaker sensitivity to the phase-shifting effects of light may have some implications for the role of BDNF in the circadian regulation of the SCN pacemaker by photic signals from the retinohypothalamic tract.


To determine the role of cytokines in the nervous system, we examined the effect of interleukin-12 (IL-12) on the nerve regeneration of mouse superior cervical ganglion cells (SCG). IL-12 enhanced the neurite outgrowth in a concentration-dependent manner. Immunocytochemical studies demonstrated the expression of IL-12 receptors in neuronal bodies and neurites. The mRNA expression of IL-12 receptors in SCG cells was confirmed by reverse transcription-polymerase chain reaction. Our data demonstrated the presence of IL-12 receptors in sympathetic neurons and suggest that IL-12 plays an important role in neuronal regeneration.


Recently, the gene called DAAO was reported to be associated with schizophrenia in the French Canadian populations. Here, we report a result obtained in the study of our large collection of 547 schizophrenia cases and 536 controls in the Chinese population. Six single-nucleotide polymorphisms (SNPs) were genotyped at and around the DAAO locus, covering a 10-kb region entirely encompassing the complementary DNA sequences of DAAO. We found statistically significant differences in allele distributions on one marker: SNP rs3741775 (P = 0.0000001). In the haplotype analysis based on the information of linkage-disequilibrium block across this gene locus, we demonstrated a highly significant association between schizophrenia and a DAAO haplotype (P = 2.0173 X 10^{-21}), which therefore provides an independent statistical support for association of the DAAO gene with schizophrenia and indicates that the DAAO gene may play a significant role in the etiology of schizophrenia in the Han Chinese.


Herpes simplex virus encephalitis (HSVE) causes elevated morbidity and mortality despite antiviral treatment. Virus-independent mechanisms may perpetuate brain damage. Matrix metalloproteinases (MMPs) target extracellular matrix components. This study describes the
protein and mRNA expression of MMP2 and MMP9 in experimental HSVE in the short and long term. Ten SJL-NBOM mice were infected with neurovirulent HSV-1 and compared with nine controls. The presence of MMP2 and MMP9 in brain tissue was analyzed with sodium-dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gelatin zymography and mRNA expression of MMP2 and MMP9 with quantitative real-time PCR at days 7, 21 and 180 post-inoculation. Infected animals had a significantly elevated gelatinolytic activity of MMP2 at all time points, and of MMP9 at days 21 and 180. Increased presence of MMP2 and MMP9 in chronic HSVE may contribute to ongoing damage. Inhibition of MMP2 and MMP9 might be a suitable target for therapeutic intervention.


http://www.sciencedirect.com/science/article/B6T0G-3S83B02-3/2/525255694a13783826e3572a2ba6f42d

In the brain tissue of 21 mice infected with herpes simplex virus type 1 (HSV-1) strain F we determined the expression of immunologic nitric oxide synthase (iNOS) as a potential mediator of neuronal injury with a semiquantitative reverse transcription polymerase chain reaction. Viral burden in brain tissue was quantitated with a dilutional polymerase chain reaction assay. Viral burden and iNOS-expression peaked at day 7 following infection. Thereafter viral burden declined to a low baseline value at 6 months following infection, whereas iNOS-expression was still 4-fold increased compared to baseline levels. In experimental herpes simplex virus encephalitis iNOS, as one potent mediator of neuronal injury, is upregulated in the acute and chronic disease. In future, in addition to antiviral treatment, inhibitors of iNOS might offer new therapeutic strategies in herpes simplex virus encephalitis.


http://www.sciencedirect.com/science/article/B6T0G-44J3V24-5/2/739393ac1d9466f1bb0b1d1c504ca7a

In the brain tissue of 36 mice infected with herpes simplex virus type 1, strain F, we determined the expression of inducible nitric oxide synthase (iNOS) with semiquantitative reverse transcription polymerase chain reaction. The viral burden was quantitated by polymerase chain reaction. Nitric oxide, induced by iNOS, may contribute to neuronal cell damage following virus infection. As the experimental therapeutic strategy in herpes simplex virus encephalitis (HSVE), we used: N-nitro–arginin (L-NA), a selective inhibitor of iNOS; and combination therapies of either methylprednisolone/acyclovir or L-NA/acyclovir. The viral burden peaked in acute disease, and then returned to a low baseline value, except in untreated controls. The expression of iNOS mRNA was suppressed by L-NA and by acyclovir/corticosteroids. iNOS inhibition may provide an additional therapeutic strategy targeted specifically to suppress iNOS expression as a potential secondary mechanism of tissue damage in acute and chronic HSVE.

Mika, J., Y. Li, et al. (2003). "Relationship of pronociceptin/orphanin FQ and the nociceptin receptor ORL1 with substance P and calcitonin gene-related peptide expression in dorsal root ganglion of the
Recent evidence suggests a role of prepro nociceptin/orphanin FQ (preproN/OFQ) derived neuropeptides in nociceptive signaling. Here, we examined the expression of preproN/OFQ and the nociceptin receptor ORL1 (opioid receptor like receptor 1) in the dorsal root ganglion (DRG) of the rat in relation to that of substance P (SP) and calcitonin gene-related peptide (CGRP). Double labeling in situ hybridization revealed a constitutive expression of preproN/OFQ in a distinct minor subpopulation of very small DRG neurons with no evidence for coexpression with either SP or CGRP. However, a major subpopulation of the preproN/OFQ-positive neurons showed direct juxtaposition to SP and CGRP containing neurons. ORL1 was abundantly expressed with a high degree of coexpression with SP (72%) and CGRP (82%) suggesting that N/OFQ may presynaptically modulate primary sensory nociceptive signaling. The DRG cell line F11 was found to express preproN/OFQ, but not ORL1, and, therefore, is well suited to study the mechanisms of N/OFQ gene regulation in vitro.


The effects of leukemia inhibitory factor (LIF) on the expression of neurotransmitter synthetase and neuropeptide mRNAs in cultured rat cortical neurons were examined by reverse transcription-polymerase chain reaction. Nociceptin mRNA expression was increased by treatment with 20 or 80 ng/ml LIF for 24 h, but choline acetyl transferase, glutamic acid decarboxylase, enkephalin, dynorphin, substance P, somatostatin and galanin mRNA expression were not altered by LIF. These observations indicated a specific effect of LIF on nociceptin gene regulation in cultured cortical neurons.


In the brain, metallothionein (MT)-III exhibits a free radical scavenging activity. Here we examined the expression of MT-III mRNA in the basal ganglia of 6-hydroxydopamine (6-OHDA)-lesioned hemi-parkinsonian rats and its regulation by levodopa. The level of MT-III mRNA was significantly decreased in the striatum of 6-OHDA-lesioned side. Levodopa treatment significantly increased the expression of striatal MT-III mRNA in the non-lesioned side, but showed no significant effect in the 6-OHDA-lesioned side. These results suggest that the regulation of MT-III mRNA may be related to the progressive degeneration in parkinsonism.

The glutamate transporter plays an essential role in regulating glutamate levels in the synaptic cleft. It has been postulated that the dysfunction of GLT-1, one subtype of glutamate transporter, may be etiologically related to amyotrophic lateral sclerosis (ALS). Two alternative splicing forms of GLT-1 messenger RNA (mRNA) were found in the cervical spinal cord of five ALS patients and three controls. Analysis with reverse transcription-polymerase chain reaction (RT-PCR) showed that the shorter mRNA was a result of exon 8 skipping. A truncated transcript containing an intronic sequence at the 3’ end of exon 7 was also demonstrated. However, the incidence of both alternative mRNAs was not different between the five ALS patients and three controls. Interestingly, the mRNA were also found in the cerebral cortex of a control subject. These results suggest that alternative splicing forms of GLT-1 mRNAs do not play a pathogenetic role in ALS but rather a physiological one in the normal spinal cord and brain.


Recently, mutations in the GABAA-receptor [gamma]2 subunit (GABRG2) gene were identified in two families with generalized epilepsy with febrile seizures plus (GEFS+) and two families with childhood absence epilepsy (CAE) and febrile seizures (FS). We tested the hypothesis that genetic variations in the GABRG2 gene confer susceptibility to FS in the Japanese population. We performed a systematic search for mutations in 94 unrelated Japanese patients with FS and detected six variants (-158C>T, 315C>T, 588T>C, IVS5-55C>T, IVS7+20G>A, and IVS7-141T>A). No non-synonymous mutation was detected. We genotyped three exonic polymorphisms and performed a case control study and a transmission disequilibrium test using 55 independent complete trios with FS and 106 control subjects. None of these polymorphic alleles were significantly associated with FS. Our results indicate that genomic variations of GABRG2 are not likely to be substantially involved in the etiology of FS in the Japanese population.


The neuropeptide Y-induced vasoconstriction of human cerebral arteries is mediated by the neuropeptide Y Y1 receptor. We conclude this on the basis of our results from: (1) in vitro studies on neuropeptide Y agonists. Neuropeptide Y and pro34NPY caused potent and long-lasting contractions of human cerebral arteries, while NPY 13-36 had no contractile effect at all on the vessels tested; (2) in vitro studies using the selective Y1 receptor antagonist BIBP3226 which in increasing concentrations (10-9-10-6M) caused a parallel shift to the right of the neuropeptide Y concentration-response curve without change of the maximum contractile response (pA2 value 8.38+/-.10); and (3) with reverse transcriptase-polymerase chain reaction (RT-PCR) we detected specific mRNA for a neuropeptide Y Y1 receptor in human pial and human middle cerebral arteries using three forward primers and one reverse primer.
Vascular endothelial growth factor (VEGF) is an endothelial cell-specific antigen and angiogenic factor that plays a role in angiogenesis. We analyzed the expression of four VEGF mRNA isoforms in meningiomas. Among 35 meningiomas, 11 came from patients who underwent complete (n=4) or partial (n=7) preoperative embolization. Northern blotting revealed markedly elevated expression of total VEGF mRNA in meningiomas compared with normal brain tissues. Semiquantitative reverse transcription-polymerase chain reaction revealed that the four isoforms are expressed with relative levels of VEGF121>165>206=189 in all samples. However, the VEGF121 and 165 isoforms were significantly upregulated in samples from patients who underwent partial preoperative embolization. The diffusible VEGF121 isoform may be important for vascularity and edema formation in meningiomas.

Alzheimer's disease is a complex neurodegenerative disorder, characterized by cognitive decline and distinctive neuropathology. Using large extended families with multiple affected, we found that three markers on chromosome 12 were linked with late-onset Alzheimer's disease. These markers were downstream from the gene for alpha-2 macroglobulin. It is likely that multiple genes will be identified either as risk factors or as causative agents for late-onset Alzheimer's disease.

Intercellular adhesion molecule-1 (ICAM-1) is implicated in the pathogenesis of ischemic cardiovascular disorders, including cerebral ischemia. A common polymorphism of the ICAM-1 gene (K469E) has been recently reported. In this case-control study, we evaluated the association between this polymorphism and vascular dementia (VD) by studying 107 patients affected by probable VD and 115 age- and sex-matched controls. The frequency of the EE genotype was significantly higher in VD patients than controls (P=0.009). Logistic regression analysis indicated that the presence of the EE genotype significantly increased the risk of VD (odds ratio 3.25, P=0.024). Our findings support the hypothesis that ICAM-1 plays a role in the physiopathology of ischemic cerebrovascular disorders and suggest that genetic polymorphisms of ICAM-1 might be clinically important in the development and progression of neurodegenerative diseases.

http://www.sciencedirect.com/science/article/B6T0G-3TJC7K5-V/2/be3e465042be2a2f4aface5df02daa6e

The 5-HT1D receptor is a potential target of anti-migraine drugs, and here its genes were cloned from chimpanzee, gorilla and rhesus monkey, via polymerase chain reactions with their genomic DNAs and the primers designed from the 5' and 3' untranslated regions of the human receptor. Direct sequencing of the polymerase chain reaction (PCR) products revealed high degrees of identity between their deduced amino acid sequences (the chimpanzee, gorilla and rhesus monkey) and that of human, differing by two, four and 11 residues, respectively. The binding properties of the receptors, as expressed in human embryonic kidney 293 cells, were compared to those obtained with the human and guinea pig receptors, the latter differing by 33 residues from the human receptor. Standard serotonergic ligands including several indoles, ergots and methiothepin bound all the cloned primate and guinea pig receptors with comparable, low nanomolar affinities, leading to high correlation coefficients among their Ki values. R(+)-8-Hydroxydipropylaminotetralin, on the other hand, bound the human receptor with the affinity higher than those for the primates and guinea pig receptors. This indicates that certain chemical templates may differentiate the molecular divergences among the 5-HT1D receptors of various animal species, and the use of the non-human primates will be beneficial for pharmacological characterizations, more relevant to the human receptor, of future novel ligands for the 5-HT1D receptor, which are potential anti-migraine drugs.


http://www.sciencedirect.com/science/article/B6T0G-3YB9Y52-1N/2/712daefdf2e9c7d9cb0557419f6f0554

Acetylcholine receptor-inducing activity (ARIA) is a glycoprotein initially purified from chick brain based on its ability to increase the synthesis of acetylcholine receptor (AChR). We used reverse transcription-polymerase chain reaction (RT PCR) to obtain a partial pro-ARIA cDNA clone from methonine-1 to serine-358 including the full functional sequence of ARIA. Northern blot analysis of mRNAs from the embryonic chick brain and muscle showed a transcript with a size of ~7.5 kb. The cloned cDNA was subcloned into an eukaryotic expression vector and stably transfected into human embryonic kidney 293 cells. The conditioned medium of the transfected cells was found to increase the level of transcript encoding for the [alpha]-subunit of AChR by ~4.4-fold, but not for acetylcholinesterase (ACNE), in the cultured chick myotubes.


http://www.sciencedirect.com/science/article/B6T0G-4DXWMBT-4/2/9589ed5318dfa311401f83d831a54732

Recently, proteolipid protein 1 (PLP1) has been identified as downregulated in schizophrenia by quantitative PCR and other technologies. In this work we attempted to investigate the role of PLP1 in the etiology of schizophrenia using a family based association study in 487 Chinese Han
family trios. The TDT for allelic association demonstrated that, in male, a weak association was
detected in SNP rs475827 with \( p = 0.0294 \), suggesting that the genetic polymorphisms within
PLP1 in male are likely to confer an increased susceptibility to schizophrenia in the Chinese
population.

Saito, S., M. Ikeda, et al. (2005). "No association was found between a functional SNP in ZDHHC8 and

http://www.sciencedirect.com/science/article/B6T0G-4DPCB36-2/2/580b439ad197837eee306cf467e0291d

ZDHHC8 is a new and attractive candidate for a schizophrenia-susceptibility factor. First, several
lines of linkage studies showed that 22q11, on which ZDHHC8 is located, is a "hot" region.
Second, fine linkage disequilibrium mapping revealed a significant association around ZDHHC8.
Moreover, a very recent study reported that one single nucleotide polymorphism (SNP: rs175174)
in ZDHHC8 might affect the splicing process, the ZDHHC8 knock-out mice showed the gender-
specific phenotype, and the transmission disequilibrium test (TDT) using this SNP also showed
significant association with human female schizophrenia. Thus, we attempted a replication study
of this SNP using relatively large Japanese case-control samples (561 schizophrenics and 529
controls). No association was found between schizophrenia and controls even after dividing
samples by gender. Because our sample size provided quite high power, ZDHHC8 may not play
a major role in Japanese schizophrenia. And our results did not support the gender-specific effect
of this SNP.


http://www.sciencedirect.com/science/article/B6T0G-3TJC7K5-1N/2/332a3a6659e3b1703168546cc681cda0

The primary structure of serotonin N-acetyltransferase (arylalkylamine N-acetyltransferase, AA-
NAT: the rate-limiting enzyme in melatonin synthesis) in the mouse retina was deduced from the
cDNA nucleotide sequence. The deduced protein consisted of 205 amino-acid residues with
sequences highly conserved in AA-NATs of vertebrates, and was 96% identical to rat AA-NAT.
Northern blot analysis of mouse retinal mRNA showed two obvious bands, of 1.5 kb and 4.5 kb in
length. The levels of both transcripts were low at day and high at night, but the night-to-day ratios
were <2. These findings suggest that the expression mechanism of AA-NAT transcripts in the
mouse retina may be different from those in other mammals, where a single transcript of AA-NAT
is normally observed in Northern blots.

Salsano, E., B. Pollo, et al. (2004). "Expression of MATH1, a marker of cerebellar granule cell

http://www.sciencedirect.com/science/article/B6T0G-4DBKH8W-D/2/006ddc8c8cfc20c1f85dde2f3d631cbc

In order to look for genetic markers helpful for the biological risk stratification of medulloblastomas
(MBs) we assayed by real-time PCR expression levels of the following genes: MATH1, encoding
a critical transcription factor for the differentiation of cerebellar granular cells (CGCs); PEDF, that
encodes a trophic factor for CGCs and is located in a region of frequent allelic imbalance in MBs; and BIRC5, encoding the antiapoptotic protein survivin, usually overexpressed in malignancies. Expression levels of TRKC, higher in MBs with a more favorable prognosis, were also studied. Twenty-three patients were considered: MATH1 expression was strong in 14/23 and undetectable in the others. PEDF was up-regulated in 8/23, TRKC in 9/23, and BIRC5 in 23/23. MATH1 expression was significantly correlated with adult age (p < PEDF and TRKC up-regulation (p < MATH1 is selectively expressed in the external germinal layer (EGL) of the cerebellum. Thus, MATH1 expression identifies a subgroup of MBs that derive from the EGL and arise during adult age into cerebellar hemispheres. MATH1 mRNA-positive MBs express high levels of PEDF and show a trend towards longer survival, in agreement with increased expression of TRKC. BIRC5 expression, which is strong in all MBs and absent in normal cerebellum, lacks any prognostic value but could be explored for selective targeting of therapeutic factors to MBs.


http://www.sciencedirect.com/science/article/B6T0G-3WRJPMH-5/2/e03d1448f010ed14e942ecfa2b609358

Previously, we reported that various levels of acetylcholine (ACh), currently known as a neurotransmitter, are detectable in the blood of several mammals including humans and that most blood ACh originates from T-lymphocytes. To investigate whether ACh in the blood acts on lymphocytes and participates in the modulation of immune responses, we have analyzed the expression of mRNA for muscarinic (Ms) ACh receptor subtypes and nicotinic (Nc) ACh receptor subunits using reverse transcription-polymerase chain reaction (RT-PCR) methods. The cells tested were human peripheral mononuclear leukocytes (MNLs) from seven healthy donors and eight leukemic cell lines, as models of lymphocytes. We detected mRNA expression for various neuronal Nc receptor subunits and Ms receptor subtypes in all of the MNL samples and in all of the cell lines tested. However, the expression pattern of mRNA for neuronal Nc receptor subunits ([alpha]2-[alpha]7 and [beta]2-[beta]4) and Ms receptor subtypes (m1-m5) varied among the individuals and cell lines. No expression of mRNA for three muscle-type Nc receptor subunits ([alpha]1, [beta]1 and [epsi]1) was observed in the MNLs and cell lines. These results indicate that both neuronal-type Nc and Ms ACh receptors are present on the surface of lymphocytes.


http://www.sciencedirect.com/science/article/B6T0G-4DXBTX8-2/2/7a80eadf93f76fddba5090c40466bcecc

Mortality and morbidity rates remain high among patients with herpes simplex virus encephalitis (HSVE). Chemokine-mediated recruitment and activation of leukocytes to focal areas of viral CNS infection are crucial steps in antiviral response and clearance. However, the inflammatory reaction and cellular antiviral response may enhance collateral damage to neurons and account for chronic progressive brain damage. We identified a specific mRNA expression of the interferon-gamma-inducible chemokines (CXCL9, CXCL10 and CXCL11), and RANTES (CCL5) in the acute course and long-term of experimental HSVE. This pattern was substantially altered by anti-viral and anti-inflammatory treatment. Our findings indicate a pivotal role of these chemokines in the immunopathogenesis of HSVE.
Brain insults, including cerebral ischemia, can alter glutamate receptor subunit expression in vulnerable neurons. Understanding these post-ischemic changes in glutamate receptors could enhance our ability to identify specific, novel neuroprotective compounds. Reverse transcription-polymerase chain reaction (RT-PCR) amplification was used to quantify the altered expression of the N-methyl--aspartate (NMDA) NR2A, NR2B and NR2C subunits relative to one another in rat hippocampal slices in resistant and vulnerable regions following in vitro oxygen-glucose deprivation. Ninety minutes after re-oxygenation and return to 10 mM glucose, there was a significant increase in the expression of NR2C relative to NR2B and NR2A in the slice as a whole, as well as in the selectively vulnerable CA1 region and the resistant CA3 and dentate gyrus regions.


Our previous research has demonstrated that androgen treatment during the perinatal period increases granulin (grn) precursor mRNA levels in the neonatal rat hypothalamus. To elucidate whether exogenous estrogen increases grn mRNA in the neonatal hypothalamus, expression of grn gene in the neonatal hypothalamus was studied by the competitive reverse transcription-polymerase chain reaction method. At 6 and 10 days of age, grn gene expression was significantly increased in the hypothalamus of pups whose dam has been dietarily administrated ethinyl estradiol from day 15 of gestation to the day of sampling. The subcutaneous injection of estradiol benzoate to neonatal rats at 2 days of age significantly increased grn gene expression on day 10. It was shown that estrogen, as well as androgen, was able to induce grn gene expression in the neonatal hypothalamus.


Receptor gated Ca2+ entry has been associated with transient receptor potential (TRP) proteins encoded by several different genes. Here, we compare expression of mRNA for TRP isoforms encoded by genes TRP1-6 in the rat substantia nigra and whole brain. The substantia nigra and the whole brain expressed mRNA predominantly for TRP3 and TRP6. The levels of TRP1, 2, 4 and 5 were very low in both. The TRP6 mRNA levels in substantia nigra and the whole brain were comparable while those for TRP3 were significantly lower in substantia nigra than in the whole brain. Thus substantia nigra differs from the whole brain in its TRP expression.
Noradrenaline (NA)- and neuropeptide Y (NPY)- containing cell bodies were found to occur in high numbers (>75% of all cells were positive) in the human superior cervical ganglion and distributed homogeneously throughout the ganglion and showed co-localisation. A few cell bodies were VIP-immunoreactive (-ir) less than 5% but none of them showed NOS-, CGRP- or SP-ir. Receptor mRNA expression was studied with RT-PCR. Total RNA from the superior cervical ganglion was successfully extracted. By using appropriate sense and antisense oligonucleotides designed from the published human sequences, we could show the presence of mRNA for the human NPY Y1, NPY Y2 and VPAC1 receptors but not CGRP1 receptor mRNA.

In this study we have characterized the nucleotide sequence of the cDNA for the growth hormone receptor (GHR) and examined the effects of morphine on the gene transcripts for GHR as well as GH binding protein (GHBP) in the male rat hippocampus and spinal cord. Using reverse transcription-polymerase chain reaction followed by cloning and sequencing, we found that the entire coding region of the GHR mRNA in the spinal cord is identical to that previously described in liver. A similar observation was made for the partially sequenced GHR cDNA from hippocampus. Northern blot analysis showed that in both tissues the levels of the transcripts for both GHR and GHBP were significantly decreased 4 h after a single dose of morphine. After 24 h the level of both transcripts did not significantly differ from that of control animals. This result indicates that the opiate does not only affect the receptor protein as shown earlier by binding studies, but also reduces the expression or turnover of the GHR as well as GHBP at the transcription level.

Neuromodulative free D-serine is present in mammalian brain, and localized to type-2 astrocytes in culture. D-amino acid oxidase (DAO) is a flavoenzyme that catalyzes D-amino acids. We examined the DAO gene expression in cultured rat astrocytes by reverse transcriptase-polymerase chain reaction. We established a method to prepare highly purified culture of type-1 and type-2 astrocytes from any brain region. This method utilizes combination of cell type specific separation by shaking and subsequent purification by immunopanning or treatment with cytosine arabinoside. We detected higher DAO gene expression in type-1 astrocyte cultures from cerebellum than that from cerebral cortex. In cerebellum, we observed higher DAO expression in
type-1 astrocyte cultures than that in type-2. We also revealed that DAO expression in C6, corresponding to type-1 astrocyte, was higher than that in CG-4 derived type-2 astrocytes.


http://www.sciencedirect.com/science/article/B6T0G-3WXP07W-C/2/3fd899e50c17809d3a78ca9559f0e7cb

The ability of homogenates from Alzheimer and control brains to inhibit formation of thiobarbituric acid reactive products (TBAR) induced by free radicals was compared. The assay for TBAR was modified by adding 1% sodium dodecyl sulfate (SDS) to prevent chromogen adsorption by biological matrices, and by extending the incubation time. The inhibitory activities required smaller equivalents of Alzheimer brain homogenates than control homogenates. Similar inhibitory activities were seen in homogenates from amygdala, temporal cortex and cerebellum. The inhibitory activities were similar in brain homogenates from individuals with different apolipoprotein E status. These results indicate that Alzheimer brain tissue has either increased content of free radical scavengers or is more sensitive to free radical attack than control brains.


http://www.sciencedirect.com/science/article/B6T0G-3YCF4WM-T/2/5b41af3f483b8876e1d88cea10ce90d

We have screened a large sample of patients with sporadic late-onset dementia of the Alzheimer type (DAT) and age-matched controls for a mitochondrial tRNAGln variant previously reported to be associated with increased risk of developing Alzheimer's disease (AD). The frequency of an Ava II site gain was determined by restriction analysis of a PCR-amplified mitochondrial DNA product. One of 155 DAT cases and four of 105 age-matched controls carried the variant. Both the affected and control frequencies are statistically different from those previously reported. The mitochondrial lineage of those individuals harboring the variant was determined by sequencing a short region of the hypervariable mitochondrial D-loop. The affected individual and three of the four controls carrying the Ava II variant belong to the same mitochondrial lineage previously reported to be associated with AD.


http://www.sciencedirect.com/science/article/B6T0G-4DKKYV4-5/2/90e2f71c5217e64da22435e1beed292b

Apoptosis is thought to play a role in neuronal pathology in schizophrenia. Recently, the GSN gene was reported to have anti-apoptotic properties. In a genome-wide expression analysis on schizophrenia, GSN was also found to be significantly down-regulated in schizophrenia. All the hints suggest that GSN is a novel candidate gene in occurrence of schizophrenia. In this work, we genotyped 3 SNPs around the GSN locus in 493 sets of the Han Chinese trio sample using allele-specific PCR. A weak association or a marginally positive result was detected (0.05 for P-value of the overtransmitted haplotype and 0.02 for a global P-value).


Schizophrenia is a debilitating mental disorder. The TP53 tumor suppressor gene, encoding a phosphoprotein, is a key element in maintaining genomic stability and cell apoptosis. Recently, reduced risk of cancer in patients of schizophrenia has been reported. Some evidence also suggests the possible implication of TP53 in neurodevelopment. In order to examine the role of the TP53 gene in the pathogenesis of schizophrenic disorders, we investigated the genetic association between a functional polymorphism rs1042522 and schizophrenia by sequencing the fragment covering 72Pro> Arg in 701 cases and 695 controls in this work. In addition, we studied two other SNPs rs2078486 and rs8064946 by allele-specific PCR in the same samples. Though rs1042522 and rs8064946 did not show positive association with schizophrenia, we did observe statistically significant differences on SNP rs2078486 (P-value = 0.029; OR = 1.21; 95% CI 1.02-1.42) and on haplotype CAC (P-value = 0.0068; OR = 1.36; 95% CI 1.09-1.70). These results demonstrated that TP53 might play a role in susceptibility to schizophrenia.


http://www.sciencedirect.com/science/article/B6T0G-3W788DW-3/2/157033e68d7a5849e58e85f085b0a0

Activity-dependent neurotrophic factor (ADNF) was recently isolated from conditioned media of astrocytes stimulated with vasoactive intestinal peptide (VIP). ADNF provided neuroprotection at femtomolar concentration against a wide variety of toxic insults. A nine amino acid peptide (ADNF-9) captured with even greater potency the neuroprotective activity exhibited by the parent protein. Utilizing Northern and Western blot analyses, it was now shown that ADNF-9 increased the expression of heat shock protein 60 (hsp60) in rat cerebral cortical cultures. In contrast, treatment with the Alzheimer's toxin, the [beta]-amyloid peptide, reduced the amount of intracellular hsp60. Treatment with ADNF-9 prevented the reduction in hsp60 produced by the [beta]-amyloid peptide. The protection against the [beta]-amyloid peptide-associated cell death provided by ADNF-9 may be mediated in part by intracellular increases in hsp60.