



DOTTORATO INTERNAZIONALE  
IN NEUROFARMACOLOGIA

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# IL PROMETEO



Bollettino di Dottorato

Direttore: Prof. Filippo Drago

## PUBBLICAZIONI DEL DOTTORATO INTERNAZIONALE IN NEUROFARMACOLOGIA (2009 - 2010)

*Neuropsychopharmacology*. 2009 Feb;34(3):593-606. Epub 2008 Jun 25.

### **Anxiolytic effects in mice of a dual blocker of fatty acid amide hydrolase and transient receptor potential vanilloid type-1 channels.**

Micale V, Cristino L, Tamburella A, Petrosino S, Leggio GM, Drago F, Di Marzo V.

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The endocannabinoid-inactivating enzyme, fatty acid amide hydrolase (FAAH), and the transient receptor potential vanilloid type-1 (TRPV1) channel are new targets for the development of anxiolytic drugs. We studied the effect on anxiety-like behavior in the elevated plus maze of a dual FAAH/TRPV1 blocker, N-arachidonoyl-serotonin (AA-5-HT). In male C57BL/6J mice, acute intraperitoneal administration of AA-5-HT (0.1-2.5 mg/kg) increased both the time spent and the number of entries in the open arm, while being inactive at the highest dose tested (5 mg/kg). AA-5-HT was more potent than selective blockers of FAAH or TRPV1 (URB597 and SB366791, respectively). In male Swiss mice, AA-5-HT had to be administered chronically to observe an anxiolytic effect at an intermediate dose (2.5 mg/kg), the highest dose (5 mg/kg) being anxiogenic, and 1 mg/kg being ineffective. In both strains, the anxiolytic effects of AA-5-HT were paralleled by elevation of brain endocannabinoid levels and were reversed by per se inactive doses of the cannabinoid receptors of type-1 (CB(1)) receptor antagonist AM251, or the TRPV1 agonist, olvanil. Immunohistochemical localization of CB(1) and TRPV1 receptors was observed in mouse prefrontal cortex, nucleus accumbens, amygdala, and hippocampus. Simultaneous 'indirect' activation of CB(1) receptors following FAAH inhibition, and antagonism at TRPV1 receptors might represent a new therapeutic strategy against anxiety.

*Br J Ophthalmol*. 2009 Feb;93(2):254-7. Epub 2008 Oct 31.

### **A water-soluble carbon monoxide-releasing molecule (CORM-3) lowers intraocular pressure in rabbits.**

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**BACKGROUND:** Carbon monoxide-releasing molecules (CORMs) are a novel group of substances that are capable of modulating physiological functions via the liberation of CO.

**AIMS:** This study was undertaken to investigate the effects of CORM-3, a water-soluble CO-releasing agent, on two rabbit models of ocular hypertension.

**METHODS:** Ocular hypertension was induced by injecting alpha-chymotrypsin in the rabbit eye. The dose-response effect of CORM-3 on IOP was assessed by topical administration of the drug (0.001, 0.01, 0.1 and 1%). Ocular hypertension was also obtained by weekly subconjunctival injection of betamethasone, and animals were treated topically with CORM-3. A group of animals in both models was treated with the inactive form of the drug (iCORM-3).

**RESULTS:** CORM-3 induced a dose-dependent reduction in IOP in rabbits treated with alpha-chymotrypsin. A similar reduction in IOP was observed in rabbits with betamethasone-induced ocular hypertension treated with the drug. Treatment with the iCORM-3 had no effect on IOP in both models.

**CONCLUSIONS:** Treatment with CORM-3 is associated with a reduction in IOP in two different rabbit models of ocular hypertension. These results support previous findings on the effect of haem oxygenase-derived CO on IOP and suggest a direct involvement of CO system in the regulation of ocular pressure probably through the modulation of aqueous humour dynamics.



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Neurochem Res. 2009 Feb;34(2):327-32. Epub 2008 Jul 9.

### **Parkin expression profile in dopamine D3 receptor knock-out mice brains.**

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Patients affected by autosomic recessive juvenile parkinsonism (ARJP) exhibit parkin gene mutations with brain decrease in dopamine D2/D3 binding sites. To date, there are no data indicating whether the reduction in dopamine D3 receptors (DRD3) may be associated with the expression of specific parkin variants. In the present study we investigated parkin expression profile in DRD3 knock-out mice brains. RT-PCR analysis was performed to assess qualitative changes in parkin isoforms' distribution pattern and in exons' expression both in wild type controls and dopamine D3 receptor's knock-out mice. Real-time PCR was performed to quantify single exons mRNA. Results demonstrated that exons 1, 2, 4, 6, 7, 8, were more expressed in wild type compared to dopamine D3 receptor KO mice brains while some other (3, 9, 10) were lower expressed. The expression levels of exons 5, 11 and 12 did not change in both animal groups. Our analysis was confirmed by western blot, which showed that parkin protein levels were influenced by the absence of DRD3.

Neurobiol Dis. 2009 Mar;33(3):415-21. Epub 2008 Dec 11.

### **Enhanced expression of ERalpha in astrocytes modifies the response of cortical neurons to beta-amyloid toxicity.**

Carbonaro V, Caraci F, Giuffrida ML, Merlo S, Canonico PL, Drago F, Copani A, Sortino MA.

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Estrogen receptor alpha (ERalpha) is over-expressed in reactive glia under conditions of neuronal damage. To elucidate the functional significance of ERalpha overexpression, an in vitro model of reactive astrocytes with enhanced expression of ERalpha was obtained by growth in G5 culture supplement. Exposure of cortical neurons to beta-amyloid in the presence of either conditioned medium from reactive astrocytes previously treated with 17beta-estradiol (17betaE2) or transferring of 17betaE2-pretreated astrocytes, caused a greater neuroprotective effect compared to the respective control conditions, although reactive glia resulted being per se neuroprotective. Blockade of ERalpha overexpression by the ER antagonist ICI182,780 was not successful as ICI182,780 behaved as an agonist. However, complete prevention of 17betaE2 effect by ICI182,780 produced an increased sensitivity of neurons to beta-amyloid toxicity. A similar effect was observed when ERalpha knock-down was induced by siRNA. It is suggested that increased ERalpha expression in reactive glia may have a role in limiting neuronal damage.

Learn Mem. 2009 Apr 29;16(5):332-7. Print 2009 May.

### **Fatty acid amide hydrolase (FAAH) inhibition enhances memory acquisition through activation of PPAR-alpha nuclear receptors.**

Mazzola C, Medalie J, Scherma M, Panlilio LV, Solinas M, Tanda G, Drago F, Cadet JL, Goldberg SR, Yasar S.

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Inhibitors of fatty acid amide hydrolase (FAAH) increase endogenous levels of anandamide (a cannabinoid CB(1)-receptor ligand) and oleoylethanolamide and palmitoylethanolamide (OEA and PEA, ligands for alpha-type peroxisome proliferator-activated nuclear receptors, PPAR-alpha) when and where they are naturally released in the brain. Using a passive-avoidance task in rats, we found that memory acquisition was enhanced by the FAAH inhibitor URB597 or by the PPAR-alpha agonist WY14643, and these enhancements were blocked by the PPAR-alpha antagonist MK886. These findings demonstrate novel mechanisms for me-

mory enhancement by activation of PPAR-alpha, either directly by administering a PPAR-alpha agonist or indirectly by administering a FAAH inhibitor.

J Neurochem. 2009 Jun;109(6):1733-44. Epub 2009 Apr 4.

### **Prenatal stress alters glutamatergic system responsiveness in adult rat prefrontal cortex.**

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Exposure to stress during gestation alters brain development resulting in permanent alterations that may increase susceptibility to subsequent cognitive or neuropsychiatric disorders. In this manuscript we examined the effects of prenatal stress on critical determinants of the glutamatergic synapse under basal conditions as well as in response to acute stress. The main finding of this work is that gestational stress altered the responsiveness of the glutamatergic system following a challenge at adulthood. In fact, while in control animals acute swim stress enhanced the phosphorylation levels of the NMDA receptor subunits NR-1(Ser896) and NR-2B(Ser1303) as well as the phosphorylation levels of alpha calcium/calmodulin-dependent protein kinase II (Thr286), a crucial sensor of calcium fluctuations, prenatal stress prevented or attenuated such activation. This dynamic modulation is restricted to prefrontal cortex since no changes were observed in the hippocampus, in line with the different maturational profile of these brain regions. Changes were also observed in the phosphorylation of the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate subunit GluR-1(Ser831) which, however, relied on the acute stress exposure and were independent of gestational stress. These effects point to a unique interference of chronic prenatal stress with the responsiveness of specific determinants of the glutamatergic synapse at adulthood in a region specific manner. The inability to mount an homeostatic glutamatergic response to subsequent stress at adulthood may impair the normal responses of the cell to challenging situations.

Prog Neuropsychopharmacol Biol Psychiatry. 2009 Oct 1;33(7):1205-10. Epub 2009 Jul 19.

### **Antidepressant properties of the 5-HT4 receptor partial agonist, SL65.0155: behavioral and neurochemical studies in rats.**

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This study was undertaken to investigate the potential antidepressant-like properties of SL65.0155, a serotonin 5-HT(4) receptor partial agonist, in male rats of the Wistar strain tested in the forced swim test (FST), an experimental model widely used to assess antidepressant-like activity. The expression of hippocampal neurotrophic factors, such as the brain-derived neurotrophic factor (BDNF), the phosphorylated cAMP response element-binding protein (p-CREB), the B cell lymphoma-2 (Bcl-2), the Bax and the vascular endothelium growth factor (VEGF) were also evaluated by Western Blot analysis. Different groups of rats received intraperitoneally (i.p.) injections of SL65.0155 (0.1, 0.5 and 1 mg/kg), clomipramine (50 mg/kg), citalopram (15 mg/kg) or vehicle, respectively, 24, 5 and 1 h prior to the FST. Compared to the control group, SL65.0155 (0.5 and 1 mg/kg), clomipramine or citalopram injected animals showed an increased swimming and climbing behavior and reduced immobility time in the FST. Interestingly, this effect was not due to changes in the locomotor activity since all treated groups failed to show any change in motor ability as assessed in the open field test. Western blot analysis of hippocampal homogenates showed an enhancement of p-CREB, BDNF Bcl-2 and VEGF protein levels in SL65.0155 treated groups, but not in citalopram or clomipramine treated groups, used here as positive control. No change was found in Bax expression in any treated group. These findings give further support to the hypothesis that the stimulation of serotonin 5-HT(4) receptors may be a therapeutic target for depression.

*Neurobiol Dis.* 2009 Oct;36(1):70-80. Epub 2009 Jul 8.

### **Altered responses of dopamine D3 receptor null mice to excitotoxic or anxiogenic stimuli: Possible involvement of the endocannabinoid and endovanilloid systems.**

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Dopamine and the endocannabinoids, anandamide and 2-arachidonoylglycerol, interact at several levels in the brain, with the involvement of both cannabinoid CB(1) receptors and transient receptor potential vanilloid type-1 (TRPV1) channels (which are alternative anandamide receptors). Using pharmacological, immunohistochemical and analytical approaches, we investigated the response of dopamine D(3) receptor null (D3R((-/-)) mice in models of epilepsy and anxiety, in relation to their brain endocannabinoid and endovanilloid tone. Compared to wild-type mice, D3R((-/-) mice exhibited a delayed onset of clonic seizures, enhanced survival time, reduced mortality rate and more sensitivity to anticonvulsant effects of diazepam after intraperitoneal administration of picrotoxin (7 mg/kg), and a less anxious-like behaviour in the elevated plus maze test. D3R((-/-) mice also exhibited different endocannabinoid and TRPV1, but not CB(1), levels in the hippocampus, nucleus accumbens, amygdala and striatum. Given the role played by CB(1) and TRPV1 in neuroprotection and anxiety, and based on data obtained here with pharmacological tools, we suggest that the alterations of endocannabinoid and endovanilloid tone found in D3R((-/-) mice might account for part of their altered responses to excitotoxic and anxiogenic stimuli.

*CNS Neurosci Ther.* 2009 Nov 19. [Epub ahead of print]

### **TGF-beta1 Pathway as a New Target for Neuroprotection in Alzheimer's Disease.**

Caraci F, Battaglia G, Bruno V, Bosco P, Carbonaro V, Giuffrida ML, Drago F, Sortino MA, Nicoletti F, Copani A.

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Alzheimer's disease (AD) is a neurodegenerative disorder that affects more than 37 million people worldwide. Current drugs for AD are only symptomatic, but do not interfere with the underlying pathogenic mechanisms of the disease. AD is characterized by the presence of ss-amyloid (A $\beta$ ) plaques, neurofibrillary tangles, and neuronal loss. The identification of the molecular determinants underlying AD pathogenesis is a fundamental step to design new disease-modifying drugs. Recently, a specific impairment of transforming-growth-factor-beta1 (TGF-beta1) signaling pathway has been demonstrated in AD brain. The deficiency of TGF-beta1 signaling has been shown to increase both A $\beta$  accumulation and A $\beta$ -induced neurodegeneration in AD models. The loss of function of TGF-ss1 pathway seems also to contribute to tau pathology and neurofibrillary tangle formation. Growing evidence suggests a neuroprotective role for TGF-beta1 against A $\beta$  toxicity both in vitro and in vivo models of AD. Different drugs, such as lithium or group II mGlu receptor agonists are able to increase TGF-beta1 levels in the central nervous system (CNS), and might be considered as new neuroprotective tools against A $\beta$ -induced neurodegeneration. In the present review, we examine the evidence for a neuroprotective role of TGF-beta1 in AD, and discuss the TGF-beta1 signaling pathway as a new pharmacological target for the treatment of AD.

*J Chemother.* 2009 Dec;21(6):646-50.

### **The impact of prulifloxacin on vaginal lactobacillus microflora: an in vivo study.**

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The aim of this study was to evaluate the in vivo effect of a repeated-dose regimen with prulifloxacin in comparison to amoxicillin/clavulanate on vaginal lactobacillus microflora. Thirty healthy

female volunteers were treated with prulifloxacin or amoxicillin/clavulanate in this open, randomized, parallel-group, repeated-dose study. Vaginal signs and symptoms were assessed at the first doctor's Visit 0 (3 weeks prior to the start of the study), and subsequent examinations (1, 3, 5, 6, 7 and 8) (followup). Some volunteers treated with amoxicillin-clavulanate showed increased pH values and 73.3% of them had lower lactobacillus flora at Visit 3. this reduction was still present in 66.7% 3 days after the last dose and in 26.7% of subjects at the follow-up, about 7 - 13 days after the last dose. The situation was completely normalized at the second follow-up about one month after treatment stop. On the contrary, the repeated administration of prulifloxacin 600 mg tablets affected neither the pH nor the lactobacillus component of the vaginal flora in healthy fertile women. The oral administration of prulifloxacin may have advantages over some other antimicrobial agents because it preserves the normal vaginal microbiota in healthy women.

*Pharmacology.* 2010;86(3):182-8. Epub 2010 Aug 18.

### **Modulation of C6 Glioma Cell Proliferation by Ureido-Calix[8]arenes.**

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Calixarenes are synthetic macrocyclic compounds that may serve as scaffolds for biologically active molecules and have been proposed as potential anticancer agents. We synthesized a ureido-calix[8]arene carrying N-acetyl-D-glucosamine residue (compound 1) and had previously demonstrated that it inhibits C6 glioma cell migration and proliferation, with divergent mechanisms. In the present work we explored in more detail the antiproliferative effect of compound 1, comparing it to related compounds lacking either the sugar moieties (compound 2), the multiple ureido groups (compound 3) or both (compound 4). The results show that the action of compound 1 is independent of the N-acetyl-D-glucosamine residues, requires the presence of multiple ureido groups and does not seem to involve focal adhesion kinase signaling. Inhibition of proliferation is reduced by preincubation with epidermal growth factor (EGF) and vascular endothelial growth factor (20 ng/ml) with compound 1, and extracellular-related kinase phosphorylation is reduced by treatment with compound 1 in both basal and EGF-stimulated conditions, suggesting that the observed effect depends on a direct interference with growth factor signaling.

*J Ocul Pharmacol Ther.* 2010 Feb;26(1):31-5.

### **Morphine-induced ocular hypotension is modulated by nitric oxide and carbon monoxide: role of mu3 receptors.**

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**PURPOSE:**Recent findings generated from our laboratory have demonstrated the involvement of nitric oxide (NO) in morphine-induced reduction of intraocular pressure (IOP). The present study was designed to investigate the possible involvement of carbon monoxide (CO) in morphine-induced reduction of IOP and the role of mu(3) opioid receptors.

**METHODS:**New Zealand rabbits were used in this study. They were pretreated with the nitric oxide synthase inhibitor Nomega-nitro-L-arginine methyl ester (L-NAME, 1%, 30 microL), or an inhibitor of heme oxygenase (HO), zinc protoporphyrin-IX (ZnPP-IX; 0.1 mg/kg; i.v.). The same animals were then treated with morphine (100 microg/30 microL) with or without NO or CO donors administration, sodium nitroprusside (SNP) and tricarbonylchloro(glycinato)ruthenium(II) (CORM-3), respectively. A separate set of animals were pretreated with the nonselective opioid receptor antagonist, naloxone (100 microg/30 microL), or the micro(3) opioid receptor inhibitor, L-glutathione (GSH, 1%, 30 microL), in the presence of SNP or CORM-3 followed by morphine administration. IOP measurements were taken at different times after monolateral instillation of morphine.

**RESULTS:**Morphine induced a significant decrease in IOP and pretreatment with ZnPP-IX or L-NAME significantly prevented

this effect whereas administration of NO or CO donors amplified morphine-induced decrease in IOP. This effect was partially abrogated both by pretreatment with ZnPP-IX or L-NAME, and by pretreatment with naloxone and GSH suggesting that the decrease in IOP relies on exogenous NO and CO liberated from SNP and CORM-3, respectively.

**CONCLUSIONS:** We conclude that the endogenous NO/CO system and micro(3) receptors contribute to morphine-induced ocular hypotension and that the reduction of IOP elicited by morphine can be augmented by exogenous NO and CO.

*Neuropeptides.* 2010 Feb;44(1):45-51.

### **PACAP and VIP affect NF1 expression in rat malignant peripheral nerve sheath tumor (MPNST) cells.**

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In our previous study we have identified PACAP, VIP and their receptors in rat malignant peripheral nerve sheath tumor (MPNST) cells, thus showing anti-apoptotic roles. Recently it has been shown that the tumor suppressor neurofibromin, encoded by the Neurofibromatosis type 1 (NF1) gene, promotes MPNST cells sensitivity to apoptosis after serum withdrawal. In the present study we investigated whether PACAP or VIP negatively regulate NF1 expression under normal or serum-dependent pro-apoptotic culture conditions. Results indicated that serum itself significantly influenced gene and protein levels. In fact, the low NF1 levels of cells cultured in normal serum-containing medium were remarkably increased in cells switched to low- or no-serum after 24h and 48 h. Treatment with 100 nM PACAP or VIP did not affect NF1 expression when using normal amounts of serum, whereas it significantly inhibited transcript and protein levels both in low- or no-serum cultured cells. In particular, PACAP reduced NF1 levels already after 24h in low-serum cultured cells, while VIP showed a similar effect only after serum deprivation. However, both PACAP and VIP downregulated gene and protein levels within 48 h either in low-dose and serum-starved cells. Results were confirmed by fluorescence microscopy, showing that 100 nM PACAP or VIP attenuated neurofibromin cytoplasmic localization only in low- or no-serum cultured cells. The present study provides a comprehensive analysis of both neuropeptides effect on NF1 expression in normal, low- or serum-starved MPNST cells, ameliorating the hypothesis that resistance to apoptosis in serum-deprived cells might be correlated to PACAP-/VIP-induced NF1 inhibition.

*Trends Pharmacol Sci.* 2010 Apr;31(4):153-60. Epub 2010 Jan 11.

### **Transcriptional regulation of type-2 metabotropic glutamate receptors: an epigenetic path to novel treatments for chronic pain.**

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Activation of metabotropic glutamate 2 (mGlu2) receptors inhibits pain transmission at the synapses between primary afferent fibers and neurons in the dorsal horn of the spinal cord. In addition, mGlu2 receptors are found in peripheral nociceptors, and in pain-regulatory centers of the brain stem and forebrain. mGlu2 receptor agonists produce analgesia in models of inflammatory and neuropathic pain, but their use is limited by the development of tolerance. A new therapeutic strategy could be based on the transcriptional regulation of mGlu2 receptors via the acetylation-promoted activation of the p65/RelA transcription factor. "Epigenetic" drugs that increase mGlu2 receptor expression, including l-acetylcarnitine and inhibitors of histone deacetylases, have a different analgesic profile with no tolerance to the therapeutic effect after repeated dosing.

*Eur Neuropsychopharmacol.* 2011 Apr;21(4):325-32. Epub 2010 Jun 8.

### **Dopamine D3 receptor knock-out mice exhibit increased behavioral sensitivity to the anxiolytic drug diazepam.**

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Dopamine D(3) receptors (DRsD3) seem to have a pivotal role in mood disorders. Using the elevated plus maze (EPM) and the novelty-induced grooming test (NGT), we assessed the responses of DRD3-deficient (D(3)(-/-)) mice to the acute treatment (different testing time) with the anxiolytic drug, diazepam. D(3)(-/-) mice treated with diazepam (0.1 or 0.5mg/kg) exhibited a better behavioral response in the EPM than their wild type (WT). Furthermore, in D(3)(-/-) mice, but not in WT, 1mg/kg diazepam induced anxiolytic effects at all testing times. The contribution of DRsD3 in the anxiolytic effects of diazepam was confirmed by similar results obtained in EPM by using the selective DRD3 antagonist U99194A (10mg/kg) in combination with diazepam, in WT animals. D(3)(-/-) mice treated with diazepam (all doses), also showed a decrease in grooming behavior. However, the [(3)H]flunitrazepam autoradiographic analysis revealed no significant changes in D(3)(-/-) mice compared to WT, suggesting that if  $\gamma$ -aminobutyric acid receptor GABA(A) changes are involved, they do not occur at the level of binding to benzodiazepine site. These data suggest that D(3)(-/-) mice exhibit low baseline anxiety levels and provide the evidence that the DRD3 is involved in the modulation of benzodiazepine anxiolytic effects.

*Pharmacol Res.* 2010 Jun;61(6):531-6. Epub 2010 Feb 10.

### **Enhanced cognitive performance of dopamine D3 receptor "knock-out" mice in the step-through passive-avoidance test: assessing the role of the endocannabinoid/endovanilloid systems.**

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*Department of Experimental and Clinical Pharmacology, University of Catania Medical School, V.le A. Doria 6, 95125 Catania, Italy.*

Increasing evidence suggests a pivotal role of the D3 receptor (D3R) in cognitive processes and the involvement of endocannabinoid/endovanilloid signaling in the pathophysiology of neurodegenerative disorders such as Alzheimer's disease. This study was undertaken to investigate both the basal and beta-amyloid peptide 1-42 (BAP 1-42)-impaired cognitive performance of D3R((-/-)) mice, and the role therein of endocannabinoids/endovanilloids. D3R((-/-)) mice were either untreated or injected i.c.v. with 400 pMol BAP 1-42 or vehicle to be tested 14 days later in a step-through passive-avoidance paradigm. The CB(1) receptor antagonist, rimonabant (1mg/kg), or the transient receptor potential vanilloid-type 1 channel (TRPV1) antagonist SB366791, were injected intraperitoneally for 11 or 7 days. The retention test was performed 1, 7 and 14 days after the learning trial. Wild-type (WT) mice were subjected to the same procedures. D3R((-/-)) mice exhibited a better basal cognitive performance as compared to WT mice ( $p < 0.001$ ), which was reversed by TRPV1 antagonism. BAP 1-42 induced a pronounced worsening of the passive-avoidance response in all tests and in both genotypes ( $p < 0.001$ ). Rimonabant treatment never affected the cognitive performance of healthy mice, but fully counteracted BAP 1-42-induced amnesic effects in both D3R((-/-)) and WT mice only when administered for 11 days, whereas, when administered for 7 days, only transiently affected WT mice and caused more prolonged cognitive ameliorations in D3R((-/-)) mice. These results support the involvement of D3R and TRPV1 in cognitive processes and the concept that A beta peptides inhibit memory retention in mice through the involvement of endocannabinoids.

*Drug News Perspect.* 2010 Sep;23(7):430-7.

### **Therapeutic potential of nitric oxide modulation in ocular diseases.**

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Nitric oxide (NO) is an organic gas ubiquitously synthesized in mammalian tissues by NO synthase (NOS). Over the past 20 years, remarkable progress has been made in explaining the mechanism/s of NO and its functions in different biological systems. NO is produced as metabolic endproduct in specific cell life phases, and may act as a atypical neuronal messenger. NO is an important regulator of homeostatic processes in the eye and

changes in its synthesis could lead to a variety of eye diseases such as glaucoma, retinal degeneration and uveitis. Both overexpression and underexpression of NO could contribute to pathological conditions in the eye. Many works have highlighted the role of NO in a wide range of ocular diseases and recent studies from our laboratory and others have shown that a suppressive action of inducible NOS-derived NO production lowers the intraocular pressure. Indeed, from a clinical perspective, a precise regulation of NO may lead to new therapeutic options likely safer and more efficacious than currently available treatments for various sight-threatening eye diseases.

*Eur Neuropsychopharmacol.* 2010 Oct;20(10):704-13. Epub 2010 May 26.

### **The beta3 adrenoceptor agonist, amibegron (SR58611A) counteracts stress-induced behavioral and neurochemical changes.**

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These experiments were made to study the mechanisms underlying the antidepressant-like effects of the beta(3) adrenoceptor agonist amibegron (SR58611A). To this purpose, the expression levels of the hippocampal cyclic adenosine monophosphate (cAMP)-response element binding protein (CREB), brain-derived neurotrophic factor (BDNF), B-cell lymphoma-2 (Bcl-2) and Bax proteins were assessed, by using western blot analysis, in rats tested in the forced swim test (FST). Under basal conditions (no previous exposure to stressors), different groups of male Wistar rats received acutely or repeatedly (once/day for 7days) intraperitoneal (i.p.) injections of amibegron (1, 5 and 10mg/kg), the tricyclic antidepressant (TCA) clomipramine (50mg/kg), the selective serotonin reuptake inhibitor (SSRI) citalopram (15mg/kg) or their vehicles. The influence of stress-related conditions was studied in rats subjected to acute (4h) or repeated (4h/day for 7days) restraint stress, applied prior to the FST procedure. Compared to the control groups, both stressor procedures increased the immobility time in the FST and reduced hippocampal BDNF and Bcl-2/Bax ratio proteins expression, which were counteracted by amibegron (5 and 10mg/kg) treatment. Opposite effects were found in the CREB expression, since it was lower after acute and higher after repeated stress procedure, respectively. Again, these effects were reversed by amibegron treatment. Different results were obtained in animals treated with clomipramine or citalopram. Hence, it is likely that the observed behavioral effects of amibegron could be due, at least in part, to its action on hippocampal expression of neurotrophic and/or anti-apoptotic factors, supporting the hypothesis that beta(3) adrenoceptors may be a therapeutic target for the treatment of stress-related disorders.

*Int J Oncol.* 2010 Oct;37(4):927-34.

### **Protective effect of the dopamine D(3) receptor agonist (7-OH-PIPAT) against apoptosis in malignant peripheral nerve sheath tumor (MPNST) cells.**

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Emerging evidence indicates that the dopamine D(3) receptor (D(3)R) mediates protective roles both in neuronal and non-neuronal cell lines. In a previous study we proposed that neurofibromin, a large tumor suppressor protein encoded by the neurofibromatosis type 1 gene (NF1), may increase susceptibility to apoptosis after serum deprivation in malignant peripheral nerve sheath tumor (MPNST) cells, thus acting as a proapoptotic gene. In addition, it has been observed that D(3)Rs are functionally correlated to neurofibromin. In this study, we examined whether 7-OH-PIPAT, a potent dopamine D(3)R agonist, exerts an antiapoptotic role under the same culture conditions and then correlated this effect to changes in NF1 expression. Results showed that serum deprivation caused a significant reduction of cell viability (MTT assay) both after 24 and 48 h ( $p < 0.001$ ). Treatment with increasing concentrations of 7-OH-PIPAT (10(-9)-10(-5) M) induced a progressive increase in cell viability both after 24 and 48 h as compared to vehicle-treated cells, with significant changes at the highest concentrations tested (10(-6) and 10(-5) M). Consistently, at the latter two concentrations, a significant reduction in oligonucleosomes formation was observed, thus suggesting an antiapoptotic role of 7-OH-PIPAT. These results were confirmed by Hoechst 33254 nuclear staining. To investigate whether these effects were correlated to changes in NF1 transcript and protein expression, quantitative real-time PCR, Western blot and immunofluorescence analyses were performed. Results demonstrated that

the upregulation of NF1 transcripts and protein levels induced by serum withdrawal were remarkably attenuated by 10(-6) and 10(-5) M agonist treatment within 24 h ( $p < 0.01$  and  $p < 0.001$ , respectively), whereas similar effects were observed already at a lower concentration (10(-7) M) after 48 h treatment ( $p < 0.001$ ). In conclusion, these results suggest that D(3)R might mediate the protective response to serum deprivation in MPNST cells through the inhibition of NF1 gene expression, further underlying a subtle role of these receptors in MPNST development.

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### **Neurofibromin and amyloid precursor protein expression in dopamine D3 receptor knock-out mice brains.**

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Recently, it has been proposed that neurofibromin (NF1) forms a binding complex with amyloid precursor protein (APP) that interacts with the dopamine D(3) receptor (D(3)R). In the present study we investigated whether the absence of the D(3)R is correlated to modifications in the expression of both NF1 and APP. Quantitative real-time PCR analyses of both transcripts showed that NF1 mRNA levels were significantly reduced whereas APP levels were strikingly increased in D(3)R knock-out (D(3)R KO) as compared to wild type (WT) mice brains. Western blot analyses using mice whole brains produced comparable results with those obtained by mRNA measurements. Moreover, immunohistochemical analyses revealed a similar brain regional distribution of APP protein in the hippocampus, in the cerebral and cerebellar cortex of D(3)R KO mice. Conversely, hippocampal NF1 immunoreactivity did not seem to be affected by the absence of D(3)Rs. Further analyses confirmed that regional NF1 protein expression in the hippocampus was not affected by the absence of the D(3)R, whereas APP levels were still increased in this specific brain region. In conclusion, these results show the existence of a correlation among the D(3)R, NF1 and APP in mice brains and thus show the regional-specific regulation of NF1 in brains of D(3)R KO, which may contribute to gain insights into the comprehension of novel underlying mechanisms that regulate brain function.

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### **Effects of PACAP and VIP on hyperglycemia-induced proliferation in murine microvascular endothelial cells.**

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Hyperglycemia is implicated both in micro- and macro-vascular complications in diabetes mellitus. Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) are two known nonclassic regulators of angiogenesis, although their biological role on endothelial cell proliferation remains poorly defined. In the present study we hypothesized that either peptides might play an inhibitory role on hyperglycemia-induced cell growth. To this end, we investigated the effect of both PACAP and VIP on cell proliferation in murine microvascular endothelial cells (H5V) cultured both under euglycemic and hyperglycemic conditions (5 and 25 mM glucose, respectively) for 24, 48 h, 7 and 15 days. Results demonstrated that high glucose treatment induced a time-dependent increase in cell viability after 48 h ( $p < 0.05$ ), which was much more evident after 7 and 15 days ( $p < 0.001$ ). Similar effects were observed in cell proliferation, although significant changes were obtained after prolonged exposures to high glucose (7 and 15 days;  $p < 0.001$ ). The proliferative response to the glucose-enriched environment was correlated to changes in the expression of PAC1 and, to a minor extent, to VPAC2, but not VPAC1 receptors, as measured by quantitative real-time PCR. These results were further confirmed by Western blot and immunofluorescence analyses. Interestingly, 10<sup>7</sup> M PACAP or VIP treatment significantly attenuated hyperglycemia-induced increase in cell viability and proliferation after 7 and 15 days. Taken together, our findings demonstrate that both PACAP and VIP peptides exert an inhibitory activity on hyperglycemia-induced endothelial cell proliferation, thus suggesting that the effect might be mediated by PAC1 and VPAC2 receptors.