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# IL PROMETEO



Bollettino di Dottorato

Direttore: Prof. Filippo Drago

## PUBBLICAZIONI DEL DOTTORATO INTERNAZIONALE IN NEUROFARMACOLOGIA (2004 - 2007)

*Eur J Pharmacol.* 2004 Sep 13;498(1-3):111-4

### Effects of neurosteroids on ischemia-reperfusion injury in the rat retina: role of sigma1 recognition sites.

Bucolo C, Drago F.

*Department of Experimental and Clinical Pharmacology, School of Medicine, University of Catania, Italy.*

The effects of neurosteroids, 17beta-estradiol and dehydroepiandrosterone-sulfate (DHEA-S), were investigated on retinal degeneration using a rat model of ischemia-reperfusion injury. The animals were anaesthetized and retinal ischemia was induced by elevating the intraocular pressure to 120 mm Hg for 45 min.

Neurosteroids were injected intraperitoneally before ischemia and immediately after reperfusion. Retinal biochemical changes such as increase of lactate content and decrease of glucose and ATP were significantly inhibited by neurosteroids compared to the control ischemic group. The effects of 17beta-estradiol and DHEA-S were antagonized by pretreatment with the sigma1 site antagonist. These findings suggest that 17beta-estradiol and dehydroepiandrosterone-sulfate may affect the metabolic state of surviving neurons and glial cells after ischemic injury and that they act, at least in part, through involvement of sigma1 recognition sites.

*Neuroreport.* 2005 Aug 1;16(11):1203-7.

### Neuroactive steroids protect retinal pigment epithelium against oxidative stress.

Bucolo C, Drago F, Lin LR, Reddy VN.

*Department of Experimental and Clinical Pharmacology, School of Medicine, University of Catania, Catania, Italy.*

This study was undertaken to assess whether neuroactive steroids, 17beta-estradiol and dehydroepiandrosterone-sulfate, enhance survival and protect DNA of human retinal pigment epithelial cells challenged by oxidative stress, and to investigate the role of sigma1 receptors in the effects of neuroactive steroids. Retinal pigment epithelial cells were treated with various concentrations of neuroactive steroids and then exposed to hydrogen peroxide. Pretreatment with steroids resulted in significant increased viability in a dose-related manner. DNA damage induced by oxidative insult was significantly lower with steroid pretreatment. The effects of 17beta-estradiol and dehydroepiandrosterone-sulfate were antagonized by pretreatment with a sigma1 receptor antagonist. The results suggest that neuroactive steroids protect retinal cells from oxidative stress, and that this effect is mediated by sigma1 receptors.

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Neuroreport. 2006 Feb 27;17(3):287-91.

### **Sigma receptor ligands protect human retinal cells against oxidative stress.**

Bucolo C, Drago F, Lin LR, Reddy VN.

*Department of Experimental and Clinical Pharmacology, School of Medicine, University of Catania, Catania, Italy*

This study was undertaken to investigate the role of sigma receptors during the oxidative damage on human retinal pigment epithelial cells, and to assess whether sigma receptor ligands enhance survival and protect DNA of cells challenged by oxidative stress. Pretreatment with PRE-084, a sigma1 receptor agonist, resulted in significant increased viability in a dose-related manner. DNA damage induced by oxidative insult was significantly lower with PRE-084. The effects of PRE-084 were antagonized by pretreatment with sigma1 receptor antagonists (NE-100 and BD1047), but interestingly were synergized by cotreatment with BD1047 that also presented an affinity for the sigma2 receptor. The results suggest that sigma1 receptors play an important role against retinal damage, even though sigma2 receptor involvement cannot be excluded.

Eur J Pharmacol. 2006 Mar 18;534(1-3):227-32.

### **Possible involvement of nitric oxide in morphine-induced miosis and reduction of intraocular pressure in rabbits.**

Bonfiglio V, Bucolo C, Camillieri G, Drago F.

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The role of mu3 opioid receptors in morphine-induced intraocular pressure (IOP) lowering effect and miosis was evaluated in conscious, dark-adapted New Zealand white (NZW) rabbits using a masked-design study. IOP and pupil diameter (PD) measurements were taken at just before and 0.5, 1, 2, 4, 6 h after monolateral instillation of morphine (10, 50 and 100 microg/30 microl) as compared to vehicle administered in the contralateral eye. Morphine-induced ocular effects were challenged by a pre-treatment with the non-selective opioid receptor antagonist, naloxone (100 microg/30 microl), the nitric oxide synthase inhibitor, N(omega)-nitro-L-arginine methyl ester (L-NAME, 1%, 30 microl), or the non-selective mu3 opioid receptor inhibitor, reduced L-glutathione (GSH, 1%, 30 microl). Morphine induced a dose-dependent decrease in IOP and PD. Pre-treatment with naloxone totally prevented morphine-induced decrease in IOP and miosis. Ocular administration of L-NAME or GSH alone failed to affect IOP or PD of NZW rabbits. However, pre-treatment with either drugs significantly reduced, but not totally prevented ocular effects of morphine. These results suggest that biochemical mechanisms related to nitric oxide release are involved, at least in part, in morphine effects on the eye. Since the mu3 opioid receptor subtype is able to release nitric oxide and is sensitive to inactivation by GSH, it may be possible that mu3 opioid receptors are involved in morphine-induced miosis and reduction in IOP.

Eur Neuropsychopharmacol. 2006 Apr;16(3):195-203.

### **Dopaminergic drugs may counteract behavioral and biochemical changes induced by models of brain injury.**

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The dopaminergic drugs, bromocriptine, cabergoline, dihydroer-

gocryptine, pergolide and ropinirole were injected subcutaneously (s.c.) at the dose of 0.1, 0.5 and 1 mg/kg/day for 7 days into male rats of the Sprague-Dawley strain. The drug pre-treatment reverted amnesia induced in rats by hypobaric hypoxia and tested in active and passive avoidance tasks. A restoration of memory retention, as assessed in a step-through passive avoidance task, was found in animals with a 2-month brain occlusive ischemia and exposed to dopaminergic drugs for 7 days. For behavioral effects in both active and passive avoidance tests in both experimental models, the rank of relative potency was ropinirole>bromocriptine=cabergoline>pergolide>dihydroergocryptine. Spontaneous ambulation of animals with brain occlusive ischemia was increased by the higher doses of drugs. All dopaminergic drugs reduced kainate mortality rate. The rank of relative potency for this effect was ropinirole=bromocriptine=cabergoline>pergolide=dihydroergocryptine. However, no change was found in other seizure parameters (latency to first convulsion and total number of convulsions) after drug treatment. A biochemical analysis of glutathione redox index (glutathione reduced/glutathione oxidized ratio) in discrete brain areas revealed that exposure to dopaminergic drugs increased this parameter in frontal cortex, striatum and hippocampus of animals subject to hypobaric hypoxia and brain occlusive ischemia. For this effect, the relative potency rank was ropinirole>bromocriptine=cabergoline>>pergolide=dihydroergocryptine. These behavioral and biochemical findings suggest that dopaminergic drugs may counteract either behavioral or biochemical changes induced by experimental models of brain injury. This activity was found after protective activity (as found in animals pre-treated with these drugs and exposed to hypobaric hypoxia) or reversal of brain injury (as found in animals treated after 2-month occlusive brain ischemia). Their neuroprotective activity probably involves the reduction/oxidation balance of the glutathione system in the brain.

Eur J Pharmacol. 2006 Apr 24;536(1-2):200-3.

### **A novel adamantane derivative attenuates retinal ischemia-reperfusion damage in the rat retina through sigma1 receptors.**

Bucolo C, Marrazzo A, Ronsisvalle S, Ronsisvalle G, Cuzzocrea S, Mazzone E, Caputi A, Drago F.

*Department of Experimental and Clinical Pharmacology, School of Medicine, University of Catania, Catania.*

The effects of a novel N-methyladamantan-1-amine derivative [(-)-MR22] with high sigma1 receptor affinity were investigated on retinal degeneration using a rat model of ischemia-reperfusion injury. The animals were anaesthetized and retinal ischemia was induced by elevating the intraocular pressure to 120 mm Hg for 45 min. The drug was injected intraperitoneally before the ischemic damage. Retinal biochemical changes, i.e. increase of lactate content and decrease of glucose and ATP were significantly inhibited by the new and selective sigma1 receptor ligand compared to the ischemic control group. The effect of (-)-MR22 was antagonized by pre-treatment with the sigma1 site antagonist. The protective effect of (-)-MR22 on ischemic retina was confirmed by the histological analysis. These findings suggest that (-)-MR22 serves as a retinal neuroprotective agent and acts as a sigma1 receptor agonist.

Mol Pharmacol. 2006 Apr;69(4):1366-72.

### **Long-term exposure to the atypical antipsychotic olanzapine differently up-regulates extracellular signal-regulated kinases 1 and 2 phosphorylation in subcellular compartments of rat prefrontal cortex.**

Fumagalli F, Frasca A, Spartà M, Drago F, Racagni G, Riva MA.

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Antipsychotics are the drugs of choice for the treatment of schizo-

phrenia. Besides blocking monoamine receptors, these molecules affect intracellular signaling mechanisms, resulting in long-term synaptic alterations. Western blot analysis was used to investigate the effect of long-term administration (14 days) with the typical antipsychotic haloperidol and the atypical olanzapine on the expression and phosphorylation state of extracellular signal-related kinases (ERKs) 1 and 2 (ERK1/2), proteins involved in the regulation of multiple intracellular signaling cascades. A single injection of both drugs produced an overall decrease in ERK1/2 phosphorylation in different subcellular compartments. Conversely, long-term treatment with olanzapine, but not haloperidol, increased ERK1/2 phosphorylation in the prefrontal cortex in a compartment-specific and time-dependent fashion. In fact, ERK1/2 phosphorylation was elevated in the nuclear and cytosolic fractions 2 h after the last drug administration, whereas it was enhanced only in the membrane fraction when the animals were killed 24 h after the last injection. This effect might be the result of an activation of the mitogen-activated protein kinase pathway, because the phosphorylation of extracellular signal-regulated kinase 1/2 was also increased by long-term olanzapine administration. Our data demonstrate that long-term exposure to olanzapine dynamically regulates ERK1/2 phosphorylation in different subcellular compartments, revealing a novel mechanism of action for this atypical agent and pointing to temporally separated locations of signaling events mediated by these kinases after long-term olanzapine administration.

*J Neurosci.* 2006 Oct 25;26(43):10949-57.

### **DNA polymerase-beta is expressed early in neurons of Alzheimer's disease brain and is loaded into DNA replication forks in neurons challenged with beta-amyloid.**

Copani A, Hoozemans JJ, Caraci F, Calafiore M, Van Haastert ES, Veerhuis R, Rozemuller AJ, Aronica E, Sortino MA, Nicoletti F.

*Department of Pharmaceutical Sciences, University of Catania.*

Cultured neurons exposed to synthetic beta-amyloid (A $\beta$ ) fragments reenter the cell cycle and initiate a pathway of DNA replication that involves the repair enzyme DNA polymerase-beta (DNA pol-beta) before undergoing apoptotic death. In this study, by performing coimmunoprecipitation experiments on cross-linked nucleoprotein fragments from A $\beta$ -treated neurons, we demonstrate that DNA pol-beta coimmunoprecipitates with cell division cycle 45 (Cdc45) and with DNA primase in short nucleoprotein fragments. This indicates that DNA pol-beta is loaded into neuronal DNA replication forks after A $\beta$  treatment. In response to A $\beta$  the canonical DNA-synthesizing enzyme DNA pol-delta also was loaded into neuronal replication forks, but at later times than DNA pol-beta. Methoxyamine, an inhibitor of the apurinic/aprimidinic endonuclease that allows for the recruitment of DNA pol-beta during the process of base excision repair (BER), failed to affect coimmunoprecipitation between DNA pol-beta and Cdc45, indicating that DNA pol-beta loading to the replication forks is independent of DNA breaks. However, methoxyamine reduced DNA replication and ensuing apoptosis in neurons exposed to A $\beta$ , suggesting that an efficient BER process allows DNA replication to proceed up to the threshold for death. These data demonstrate that DNA pol-beta is an essential component of the DNA replication machinery in A $\beta$ -treated neurons and additionally support the hypothesis of a close association of cell cycle events with neuronal death in Alzheimer's disease (AD). Accordingly, by investigating the neuronal expression of DNA pol-beta, along with phosphorylated retinoblastoma protein and neurofibrillary changes in AD brain, we show an early involvement of DNA pol-beta in the pathogenesis of AD.

*Brain Res.* 2006 Nov 22;1121(1):207-15. Epub 2006 Oct 2.

### **Cognitive effects of SL65.0155, a serotonin 5-HT<sub>4</sub> receptor partial agonist, in animal models of amnesia.**

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Given that several data suggest the involvement of serotonergic (5-HT) system, particularly the serotonin 5-HT<sub>4</sub> receptors, in memory processes; this study was undertaken to investigate the role of serotonin 5-HT<sub>4</sub> receptors in different experimental models of amnesia in male Swiss mice or in male Sprague-Dawley rats, tested in learning and memory tasks. Amnesia was induced in mice by intracerebroventricular (i.c.v.) injection of beta-amyloid 1-42 fragment (BAP 1-42; 400 pmol/mouse) or of galanin (GAL) 1-29 (3 microg/mouse). Another group of animals was exposed to carbon monoxide (CO). Treatments were made 14 days, 15 min or 8 days prior to the learning trial of a step-through passive avoidance paradigm, respectively. Latency to re-enter the dark box appeared to be reduced in all treatment groups. Intraperitoneal (i.p.) administration of SL65.0155 (5-(8-amino-7-chloro-2,3-dihydro-1,4-benzodioxin-5-yl)-3-[1-(2-phenylethyl)-4-piperidinyl]-1,3,4-oxadiazol-2(3H)-one-mono-hydrochloride), a serotonin 5-HT<sub>4</sub> receptor partial agonist (1 mg/kg/day), for 7 days prior to the learning trial, inhibited the amnesic effect of both peptides increasing the latency to re-enter the dark box also in mice exposed to CO. In rats with ibotenate-induced lesions of the nucleus basalis magnocellularis (NBM) or prenatally exposed to methylazoxymethanol (MAM), SL65.0155 (1 mg/kg/day, i.p.) administered for 7 days, improved the learning and memory capacity in animals tested in shuttle-box active avoidance and radial maze tests. These findings give further support to the hypothesis of SL65.0155 cognition-enhancing activity across a range of tasks.

*Neurobiol Dis.* 2006 Nov;24(2):254-65.

### **Inhibition of Wnt signaling, modulation of Tau phosphorylation and induction of neuronal cell death by DKK1.**

Scali C, Caraci F, Gianfriddo M, Diodato E, Roncarati R, Pollio G, Gavigli G, Copani A, Nicoletti F, Terstappen GC, Caricasole A.

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Expression of the Wnt antagonist Dickkopf-1 (DKK1) is induced during neurodegenerative processes associated with Alzheimer's Disease and brain ischemia. However, little is known about DKK1-mediated effects on neurons. We now describe that, in cultured neurons, DKK1 is able to inhibit canonical Wnt signaling, as assessed by TCF reporter assay and analysis of beta-catenin levels, and to elicit cell death associated with loss of BCL-2 expression, induction of BAX, and TAU hyperphosphorylation. Local infusion of DKK1 in rats caused neuronal cell death and astrocytosis in the CA1 region of the hippocampus and death of cholinergic neurons in the nucleus basalis magnocellularis. Both effects were reversed by systemic administration of lithium ions, which rescue the Wnt pathway by inhibiting glycogen synthase kinase-3beta. The demonstration that DKK1 inhibits Wnt signaling in neurons and causes neuronal death supports the hypothesis that inhibition of the canonical Wnt pathway contributes to the pathophysiology of neurodegenerative disorders.

*J Endocrinol Invest.* 2006 Dec;29(11):RC35-8.

### **Comparative bioavailability of different formulations of levothyroxine and liothyronine in healthy volunteers.**

Leggio GM, Incognito T, Privitera G, Marano MR, Drago F.

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**OBJECTIVE:** To evaluate the relative bioavailability of T4 sodium and liothyronine sodium (T3), administered in single doses as oral solution (drops) and tablet forms, according to two separate study protocols.

**METHODS:** Twenty-four healthy, male volunteers were included in both studies. Two test drugs containing T4 or T3 (T4-Ibsa and T3-Ibsa, respectively) were compared to two reference drugs, ie Eutirox 100 and Ti-tre tablets, respectively. A single oral dose of 100 microg (1 ml or 1 tablet) of T4 and 20 microg (1 ml or 1 tablet) of T3 were administered with an open, randomized, crossover design. T4 and T3 serum concentrations were determined by a validated immunoassay in electro-chemo-luminescence method.

**RESULTS:** Study 1: after administration of T4-Ibsa oral solution, Cmax was 14.26±0.61 microg/dl, AUC0-t was 282.70 ±14.29 microg/dl/h, Tmax was 2.71±0.25 h. After administration of Eutirox 100 tablets, Cmax was 14.34±0.59 microg/dl, AUC0-t was 279.42±9.59 microg/dl/h and Tmax was 2.65±0.23 h. The 90% confidence interval ratios between test/reference drugs were 1.01 for AUC0-t and 0.99 for Cmax. Study 2: after administration of T3-Ibsa oral solution, Cmax was 3.19±0.25 ng/ml, AUC0-t was 44.79±2.15 ng/ml/h and Tmax was 2.31±0.25 h. After administration of Ti-tre tablets, Cmax was 3.16±0.23 ng/ml, AUC0-t was 45.19±2.19 ng/ml/h and Tmax was 2.44±0.34 h. The 90% confidence interval ratios between test /reference drugs were 0.99 for AUC0-t and 1.01 for Cmax.

**CONCLUSIONS:** The bioavailability of the two oral solutions (T4-Ibsa and T3-Ibsa oral solutions) and the corresponding tablet forms (Eutirox 100 and Ti-tre tablets) were confirmed and they can be considered bioequivalent and therapeutically interchangeable.

*Exp Eye Res.* 2006 Dec;83(6):1366-72.

### **Effects of the COOH-terminal tripeptide alpha-MSH(11-13) on corneal epithelial wound healing: role of nitric oxide.**

Bonfiglio V, Camillieri G, Avitabile T, Leggio GM, Drago F.

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It is known that alpha-melanocyte stimulating hormone (alpha-MSH) may exert anti-inflammatory effects and facilitate reparative processes in different tissues. The effective message sequence of alpha-MSH resides in the COOH-terminal tripeptide alpha-MSH(11-13). This study was undertaken to investigate the effects of topical administration of the COOH-terminal tripeptide sequence of alpha-MSH (alpha-MSH(11-13), KPV) on corneal epithelial wound healing in rabbits and the possible role of nitric oxide (NO) in these effects. The whole corneal epithelium was denuded in both eyes by mechanical abrasion. The area of the corneal epithelial defect was stained with fluorescein, photographed, and then measured before the treatment and every 12 h by a computerized software. The mean epithelial wound area and the mean percent of epithelial defect remaining at each follow-up control were compared between experimental groups. Rabbits were topically treated with KPV 1, 5 or 10 mg/ml (30 microl), two drops four times in a day, for 4 days, starting immediately after corneal abrasion, while control animals received topical phosphate-buffered saline as vehicle. In order to study the role of NO in corneal repair processes, the NO donor, sodium nitroprusside (SP, 10 mg/ml, 30 microl) was administered in both eyes, two drops four times in a day, for 4 days. The effects of KPV or SP were challenged by pre-treatment with the nitric oxide synthase inhibitor, N omega-nitro-L-arginine methyl ester (L-NAME, 10 mg/ml, 30 microl) 30 min prior to KPV or SP instillation. The mean percent epithelial defect remaining each time was significantly smaller in animals treated with KPV or SP in comparison to controls. Sixty hours later, eight out of eight (100%) corneas treated with KPV or SP were completely

re-epithelized (P<0.05) while none of the corneas treated with placebo were re-epithelized. Pre-treatment with L-NAME inhibited the facilitating effect of KPV on corneal epithelial wound healing process and totally prevented the effect of SP. Rabbit corneal epithelial cells (RCE) in culture were exposed for 1, 6 and 24 h to different KPV concentrations (0.1, 1 and 10 microM) in medium containing 15% foetal bovine serum (FBS). Cell viability was stimulated by 1 and 10 microM concentrations of the substance. Thus, KPV may facilitate corneal epithelial wound healing in rabbits with a mechanism that may involve NO disposition in corneal tissue. However, it is not known whether this mechanism is likely to depend on a direct stimulating repairing activity shared by the entire molecule of alpha-MSH.

*Basic Clin Pharmacol Toxicol.* 2007 Mar;100(3):214-6

### **Neuroactive steroids protect retinal tissue through signal1 receptors.**

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[not-structured abstract]

The term 'neuroactive steroids' has been adopted for steroids, including 17β-oestradiol and dehydroepiandrosterone- sulphate (DHEA-S), that might alter neuronal excitability through the cell surface through interaction with specific neurotransmitter receptors. It has been shown that administration of 17β-oestradiol exerts protective effects against ischaemic damage in rat retina. Recently, we demonstrated that 17β-oestradiol and DHEA-S inhibit biochemical changes induced by ischaemia injury in rat retina and that these effects were antagonized by s1 receptor blocker pre-treatment. More recently, we showed that neuroactive steroids protect retinal pigment epithelium against oxidative stress and that this effect was blocked by pre-treatment with s1 receptor antagonist. The mechanism by which neuroactive steroids exert these protective effects remains unclear. A direct interaction between neuroactive steroids and s1 receptors has been hypothesized from the finding that several steroids inhibit the binding of s1 receptor radioligands in vitro and in vivo. s1 Receptors are a unique class of non-opioid, non-phencyclidine-binding sites heterogeneously distributed in the nervous system and in peripheral organs that may serve as receptors for any, as yet unidentified, endogenous ligand. Recently, the presence of s1 receptors in rat Müller cells, rat ganglion cells and human retinal pigment epithelial cells have been demonstrated, in spite of the functional role of s1 receptors not yet being clearly determined. The present study was designed to determine whether 17β-oestradiol and DHEA-S protect rat retinal tissue against ischaemia/reperfusion damage and whether s1 receptors are involved in the mechanism of action.

*Biochim Biophys Acta.* 2007 Apr;1772(4):409-12.

### **The nature of the cell cycle in neurons: focus on a "non-canonical" pathway of DNA replication causally related to death.**

Copani A, Caraci F, Hoozemans JJ, Calafiore M, Sortino MA, Nicoletti F.

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The mechanism whereby a reactivation of cell cycle in neurons causes cell death is beginning to be identified. In cellular models of Alzheimer's disease, activation of a non-canonical pathway of DNA replication contributes to neuronal death. This pathway involves the repair enzyme DNA polymerase-beta, which is highly expressed in neurons of the Alzheimer's brain at early stages of the disease. Loading of DNA polymerase-beta into the replication forks generates a death signal, which involves the tumor suppressor p53. The increasing knowledge of the main actors of the unscheduled DNA replication in neurons will pave the way for novel therapeutic interventions in Alzheimer's disease and other neurodegenerative disorders.