XI Convegno Monotematico della SIF
Bologna, 9 settembre 2005

FINAL PROGRAM

MEETING VENUE

Aula Prodi, Complesso di S. Giovanni in Monte, Università degli Studi di Bologna,
P.za S. Giovanni in Monte 2, Bologna

SCIENTIFIC COMMITTEE

President Patrizia Romualdi, Università di Bologna
Giovanni Biggio, Università di Cagliari
Sanzio Candeletti, Università di Bologna
Giorgio Cantelli Forti, Università di Bologna
Walter Fratta, Università di Cagliari
Ottavio Gandolfi, Università di Bologna
Gian Luigi Gessa, Università di Cagliari

ORGANIZING COMMITTEE and SECRETARY

Patrizia Romualdi
Sanzio Candeletti
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SCIENTIFIC INFORMATIONS

Oral Communications

Oral communications (preferentially in English) will last 15 min including discussion.
Material for videoprojection (Power point) should be given to the Meeting Secretariat (at Aula Prodi, Complesso di S. Giovanni in Monte, Università degli Studi di Bologna, P.za S. Giovanni in Monte 2, Bologna) in the afternoon of the 8th of September or the early morning of the 9th of September).

Posters

Posters, 80 cm wide and 120 cm high, (preferentially in English) should be exhibited in the Poster Area before the beginning of the meeting (either the evening of the 8th or the early morning of the 9th of September).

REGISTRATION

Registration desk will be open from 4:00 to 7:00 p.m. of the 8th of September
and the early morning of the 9th of September.

The participation to the Scientific Meeting is free of charge.

The SIF Secretary will be open all the Meeting time.

Certificate of Attendance.
On request a certificate of attendance will be available.

At the end of the Meeting the Scientific Committee will assign a Prize as best Oral Communication and a Prize as best Poster presentation
(Medals of the Italian Society of Pharmacology).

The scientific contributors, that wish to submit a research paper to the Journal **Pharmacologyonline** concerning the topic presented to this Meeting, are invited to contact Prof. Anna Capasso (Salerno) to receive specific informations and instructions.

SCIENTIFIC PROGRAM

9:00-9:15 Opening of the meeting

P. Romualdi, President Scientific Committee
G. Biggio, President of the Italian Society of Pharmacology
G. Cantelli Forti, Dean Faculty of Pharmacy University of Bologna
W: Fratta, Meeting Scientific Committee

9:15-10:00 Plenary Lecture

Presented by Prof. Gian Luigi Gessa
Prof. Mary Jeanne Kreek (New York, USA)
ENDORPHINS, DOPAMINE, STRESS AND ADDICTION

10:00-11:15 Oral Communications

Chairpersons: W. Fratta (Cagliari), D. Parolaro (Varese)

ACTIVATION OF MULTIPLE TRANSCRIPTION FACTORS IN THE BRAIN OF CANNABINOID SENSITIZED ANIMALS.
Viganò D., Rubino T., Realini N., Castiglioni C. and D. Parolaro. DBSF, University of Insubria and Center of Neuroscience, via A. da Giussano 10, 21052 Busto Arsizio (VA), Italy

CONDITIONED PLACE PREFERENCE (CPP) INDUCED BY SALVINORIN A IN WISTAR RATS
Limonta V., Braida D., Pegorini S.,Gori E. and Sala M. Departement of Pharmacology, Chemioterapy and MedicalToxicology, University of Milan, Via Vanvitelli 32, 20129 Milan. Italy

SINGLE OR REPEATED COCAINE TREATMENT MODULATES FGF-2 EXPRESSION IN RAT DOPAMINERGIC REGION
Fumagalli F[1], Di Pasquale L[1], Leo D[2], Racagni G[1]., Perrone-Capano C[2,3]. and Riva MA[1].[1]Center of Neuropharmacology, Pharmacological Sciences Dept., University of Milan; [2]IGB, CNR, Via Pietro Castellino 111, 80131 Napoli; [3] Dept. Pharmacobiol, Univ Catanzaro "Magna Grecia", I-88021 Roccelletta di Borgia (CZ).

METHYLPHENIDATE ADMINISTRATION TO ADOLESCENT RATS DETERMINES SHORT- AND LONG-TERM CHANGES ON REWARD-RELATED BEHAVIOR AND STRIATAL GENE EXPRESSION
D. Leo [1], W. Adriani [2], D. Greco [1], M. Rea[2], U. di Porzio[1], C. Perrone-Capano [2,3], G. Laviola [2].
[1] IGB "A. Buzzati Traverso", CNR, Napoli, Italy; [2] Istituto Superiore di Sanità, Rome, Italy; [3] University of Catanzaro "Magna Graecia", Italy.

IN VIVO EVIDENCE THAT LOCAL INJECTION OF SB-277011A INCREASES CELL FIRING IN THE VENTRAL TEGMENTAL AREA (VTA)
Congestri' F, Sonntag V, Formenti F, Heidbreder C and Crespi F., Biology, Psychiatry CEDD, glaxoSmithKline, via Fleming 4, 37135 Verona, Italy

11:15-11:30 Coffee break

11:30-13:00 Oral Communications

Chairpersons :G. Biggio (Cagliari), P. Romualdi (Bologna)

PRESYNAPTIC NICOTINIC RECEPTOR SUBTYPES MODULATING NEUROTRANSMITTER RELEASE: EFFECTS OF CHRONIC TREATMENT WITH NICOTINE.

Grilli M.,* Parodi M.,* Drocco R.,* Raiteri M. *§ and Marchi M. *§ *Department of Experimental Medicine, Pharmacology and Toxicology Section, §Center of Excellence for Biomedical Research University of Genoa, Italy

SERUM PROTEOMIC ANALYSIS DURING NICOTINE SELF-ADMINISTRATION, WITHDRAWAL, EXTINCTION AND RELAPSE IN RATS

Cecconi D.[1], Tessari M.[2], Wille' D.[3], Zoli M.[4], Righetti P.G.[1], Carboni L.[2]. [1]Dept. of Agricultural and Industrial Biotechnologies, University of Verona, Verona, Italy; [2]Psychiatry CEDD, GlaxoSmithKline, Verona, Italy; [3]Dept. of Statistical Sciences, GlaxoSmithKline, Harlow, UK; [4]Dept. of Biomedical Sciences, University of Modena and Reggio Emilia, Italy;.

ACUTE AND REPEATED ADMINISTRATIONS OF MORPHINE AND PARACETAMOL: DIFFERENT EFFECTS ON NOCICEPTION, 5-HT_2 AND μ- RECEPTORS IN THE RAT BRAIN

Ruggieri V.[1], Sandrini M [2], Vitale G. [2], Pini L.A.[1] [1] Dept. of Laboratories, Sect. of Clinical Pharmacology and Toxicology [2] Dept. of Biomedical Sciences, Sect. of Pharmacology University of Modena and Reggio Emilia, Italy.

ETHANOL PROMOTES STEROIDOGENESIS IN ISOLATED HIPPOCAMPUS: EFFECTS ON GABAR FUNCTION IN NORMAL AND ADX-CX RATS

Talani G., Serra M., Pisu M.G., Murru L., Barabino E., Serra G., Zucca S., Uras R., Sanna E., and Biggio G. University of Cagliari, Department of Experimental Biology, Sect. of Neuroscience and Center of Excellence for the Neurobiology of Dependence, 09123 Cagliari.

FORCED INTERMITTENT EXPOSURE (FIE) TO ETHANOL VAPOR AND WITHDRAWAL: A NOVEL ANIMAL MODEL FOR ALCOHOLISM

R. Rimondini[1,2], W. Sommer[3], C. Arlinde[2], M . Heilig[3] 1.Dept. of Pharmacology, University of Bologna (Italy); 2. Karolinska Institute, Stockholm (Sweden); 3. NIH-AAA, NIH, Bethesda (USA)

13:00-14:00 Lunch

14:00-15:30 Poster Session and Discussion

Chairpersons: Tagliamonte A. (Siena), M. A. Riva (Milano), Ciccocioppo R.(Camerino)

15:30-16:30 Oral Communications

Chairpersons: M. Massi (Camerino), S. Candeletti (Bologna)

ROLE OF THE NOCICEPTIN/ORPHANIN FQ–NOP RECEPTOR SYSTEM IN MORPHINE TOLERANCE: PHARMACOLOGICAL AND KNOCKOUT STUDIES

Rizzi A, Marzola G, Zucchini S, *Trapella C, *Salvadori S, Regoli D, and Calo' G. Department of Experimental and Clinical Medicine, Section of Pharmacology, and *Department of Pharmaceutical Sciences and Biotechnology Center, University of Ferrara, 44100 Ferrara, Italy

NOP RECEPTOR DENSITY IS REDUCED BY CHRONIC INFUSION WITH BUPRENORPHINE IN SELECTED RAT BRAIN AREAS

Lopetuso G., Romualdi P., Candeletti S.
Dept. Pharmacology, University of Bologna, Irnerio 48, 40126 Bologna, Italy

NOCICEPTIN/ORPHANIN FQ SYSTEM: BEHAVIORAL AND MOLECULAR DIFFERENCES BETWEEN MARCHIGIAN SARDINIAN PREFERRING RATS (msP) AND WISTAR RATS.

Fedeli A., Hansson A.*, Heilig M.,*, Economidou D., Massi M., Ciccocioppo R., Dept. of Sperimental medicine and public health, University of Camerino, Italy. *Laboratory of Clinical and Translational Studies, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD.

ETHANOL-CRF-NOCICEPTIN INTERACTIONS AT GABAergic SYNAPSES IN RAT CENTRAL AMYGDALA

[1]Marisa Roberto, [1]Paul Schweitzer, [2]Roberto Ciccocioppo, [1]Samuel G. Madamba and [1]George Robert Siggins [1] The Scripps Research Institute and Alcohol Research Center, Department of Neuropharmacology, 10550 N. Torrey Pines, La Jolla, CA 92037, USA.[2] Department of Experimental Medicine and Public Health, University of Camerino, via Scalzino 3, 62032 Camerino (MC), Italy.

17:00 End (closing) of the meeting

POSTER SESSION

1. BEHAVIORAL AND NEUROCHEMICAL MODIFICATIONS IN MORPHINE SENSITIZED RATS 10-20 DAYS AND 6 MONTHS AFTER THE LAST DRUG ADMINISTRATION.

Scheggi S., Raone A., Rauggi R., Cassanelli A., Tagliamonte A.
Department of Neuroscience, University of Siena, Siena, Italy

2. THE EXPRESSION OF COX-2 AND iNOS ENZYMES BY MORPHINE WITHDRAWAL.

Cavallo F. and Capasso A. Department of Pharmaceutical Sciences, University of Salerno, Via Ponte Don Melillo (84084) Fisciano, Salerno, Italy.

3. CHRONIC BACLOFEN REVERSES, BUT DOES NOT PREVENT, MORPHINE – INDUCED MOTOR SENSITISATION.

Ricci F., Gaiardi M., Bartoletti M.
Department of Pharmacology – University of Bologna

4. DIFFERENT IMPACT OF CHRONIC TRAMADOL OR MORPHINE ON PRODYNORPHIN GENE EXPRESSION .

Lopetuso G., Landuzzi D., Candeletti S., Romualdi P.
Dept. Pharmacology, University of Bologna, Irnerio 48, 40126 Bologna, Italy

5. PRENATAL EXPOSURE TO CANNABINOID AGONIST AFFECTS MOLECULES INVOLVED IN INTRACELLULAR SIGNALING PATHWAYS.

[1]Maj P.F.; [2]Collu M.; [2]Fadda P.; [1]Racagni G.; [1]Riva M.A.
[1]Center of Neuropharmacology, Department of Pharmacological Sciences, University of Milan, via Balzaretti 9, 20133 Milan (Italy) [2]Department of Neurosciences B.B.Brodie, University of Cagliari, Monferrato, Cagliari (Italy).

6. SELECTIVE INHIBITION OF REWARD-RELATED BEHAVIOURS BY THE CANNABINOID CB1 RECEPTOR ANTAGONIST SR-141716A IN RATS.

Mattioli L[1], Economidou D[1], Perfumi M[1], Massi M[1], Cuomo V[2], Ciccocioppo R[1]
[1]Dept. of Experimental Medicine and Public Health, Univ. of Camerino, [2]Dept. of Pharmacology of Natural Substances and General Physiology, Univ. of Rome "La Sapienza

7. NEUROPHARMACOLOGICAL EVALUATION OF *CANNABIS SATIVA* ESSENTIAL OIL.

Utan A., Costa S., Guerra M.C., Scarpellini G., Speroni E.
Department of Pharmacology, Università degli Studi di Bologna, via Irnerio 48, 40126 Bologna.

8. EFFECT OF CANNABINOID CB1 RECEPTOR AGONISTS ON 5-HT EXTRACELLULAR LEVELS IN DIFFERENT RAT BRAIN AREAS.

Salis P. [1], Fadda P.[1,2], Scherma M., Fresu A.[1], Cappai A.[1], Fattore L.[2,3] and Fratta W. [1,2,3]
[1] Dept of Neuroscience and [2] Centre of Excellence "Neurobiology of Dependence, University of Cagliari; [3] Institute of Neuroscience, CNR, Cittadella Universitaria, Monserrato (Cagliari), Italy

9. EFFECTS OF A CANNABIS EXTRACT ON MOTOR ACTIVITY, ANXIETY AND NOCICEPTION IN THE RAT: INVOLVEMENT OF THE SEROTONERGIC SYSTEM.

Pini L.A.[1], Licata M.[2], Ruggieri V.[1], Vitale G [3,] Sandrini M.[3]
[1] Dept. of Laboratories, Sect. of Clinical Pharmacology and Toxicology [2] Dept. of Laboratories, Sect. of Forensic Medicine [3] Dept. of Biomedical Sciences, Sect. of Pharmacology
University of Modena and Reggio Emilia, Italy.

10. CROSS-TALK BETWEEN CB1 AND NOP RECEPTORS IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS.

Landuzzi D., Candeletti S., Romualdi P.
Dept. Pharmacology, University of Bologna, Irnerio 48, 40126 Bologna, Italy.

11. COCAINE AFFECTS NOP RECEPTOR GENE EXPRESSION IN SH-SY5Y CELLS.

Landuzzi D., Candeletti S., Romualdi P.
Dept. Pharmacology, University of Bologna, Irnerio 48, 40126 Bologna, Italy.

12. SUBCHRONIC ADMINISTRATION OF COCAINE IN THE RAT INDUCES AN UP-REGULATION OF NOCICEPTIN RECEPTOR mRNA EXPRESSION IN THE CORE OF NUCLEUS ACCUMBENS.

Zambello E.[1,2], Pilla, M.[2], Mugnaini M.[2], Fedeli A.[3], Caberlotto L.[2]
[1]Section of Pharmacology, Dept. of Medicine and Public Health, University of Verona, Italy, [2]Dept. of Biology, Psychiatry-CEDD, GlaxoSmithKline, Verona, Italy, [3]Dept. of Experimental Medicine and Public Health, University of Camerino, Italy

13. ROLE OF DIFFERENT NEUROTRANSMITTERS ON COCAINE-MEDIATED EFFECTS ON PRODYNORPHIN GENE EXPRESSION AND CREB ACTIVATION IN THE RAT BRAIN.

Di Benedetto M., D'Addario C., Candeletti S., Romualdi P.
Dept. of Pharmacology University of Bologna, Irnerio 48, 40126 Bologna, Italy

14. EFFECTS OF 3,4-METHYLENDIOXY-N-METHYLAMPHETAMINE (MDMA, 'ECSTASY') ADMINISTRATION ON THE DYNORPHINERGIC SYSTEM IN THE RAT BRAIN.

Di Benedetto M., D'Addario C., Candeletti S., Romualdi P.
Dept. Pharmacology University of Bologna, Irnerio 48, 40126 Bologna – Italy

15. ANALYSIS OF AMPHETAMINE, METAMPHETAMINE AND MDMA ("ECSTASY") IN HUMAN PLASMA AND URINE BY MEANS OF LIQUID CHROMATOGRAPHY WITH FLUORIMETRIC DETECTION.

Raggi M.A.[1], Bugamelli F. [1], Saracino M.A. [1], Mercolini L. [1], Cavallini A. [1], Baccini C. [2], Conti M. [2], Gerra G. [3]
[1]Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy; [2]Laboratory of Clinical Pharmacology and Toxicology, Hospital "S. Maria delle Croci", Ravenna, Italy; [3] National Department on Drug Policy, Rome, Italy.

16. EFFECT OF ETHANOL ON SYNAPTIC INPUT IN CEREBELLAR PURKINJE NEURONS.

Carta M.*, Mameli M.†, and Valenzuela CF†.
*Department of Experimental Biology, Section of Neuroscience and Center of Excellence for the Neurobiology of Dependence, University of Cagliari, 09123, Cagliari †Dept. of Neurosciences, U. of New Mexico HSC, Albuquerque, NM 87131, USA.

17. EFFECT OF FLUMAZENIL DURING ETHANOL WITHDRAWAL: A MOLECULAR AND FUNCTIONAL STUDY IN RAT CEREBELLAR GRANULE CELLS IN CULTURE.

Biggio F.[1], Gorini G.[1], Caria S.[1], Murru L., Sanna E. [1,2] and Follesa P.[1,2]
University of Cagliari, Department of Experimental Biology Section of Neuroscience[1], Center of Excellence for the Neurobiology of Dependance[2], 09123 Cagliari, Italy

18. ROLE OF FEEDING PEPTIDES IN ETHANOL INTAKE.

Cifani C, Polidori C, Fedeli A, Ciccocioppo C and Massi M.
University of Camerino, Department of Experimental Medicine and Public Health, Via Scalzino 3, 62032 Camerino (MC), ITALY

19. PRESYNAPTIC NICOTINIC AND mGLU RECEPTORS INTERACTION IN THE MODULATION OF NORADRENALINE RELEASE FROM FROM RAT HIPPOCAMPAL SYNAPTOSOMES.

Parodi M. *, Patti L. *, Testaquadra G. *, Raiteri M. *§ and Marchi M. *§.
*Department of Experimental Medicine, Pharmacology and Toxicology Section, § Center of Excellence for Biomedical Research University of Genoa, Italy

20. ROLE OF PRESYNAPTIC NICOTINIC AND PURINERGIC RECEPTORS IN THE MODULATION OF GLUTAMATE RELEASE FROM RAT CEREBROCORTICAL SYNAPTOSOMES.

Patti L.*, Grilli M. *, Robino F.*, Raiteri M. *§ and Marchi M. *§.
*Department of Experimental Medicine, Pharmacology and Toxicology Section, § Center of Excellence for Biomedical Research University of Genoa, Italy.

21. LINKAGE DISEQUILIBRIUM AND HAPLOTYPE ANALYSIS OF POLYMORPHISMS IN THE GABA RECEPTOR CLUSTER ON CHROMOSOME 4: ASSOCIATION STUDY.

D'Addario C.[1-2], Drgon T.[1], Romualdi P.[1-2] and Uhl GR.[1]
Molecular Neurobiology Research Branch, NIDA-IRP, NIH, DHHS 333 Cassell Drive, Baltimore, MD (USA) [1] , Dept. of Pharmacology, University of Bologna, Irnerio 48, Bologna (Italy) [2]

22. ALLELIC ASSOCIATION ANALYSIS OF THE μ -OPIOID AND CANNABINOID RECEPTOR GENES WITH HEROIN ADDICTION.

[1,2]Congiu D., [1,2]Oi A., [1,2]Serio S., [3]Agus A., [3]Loi A., [1,2]Del Zompo M, [1,2]Piccardi M.P.
[1]Section of Clinical Pharmacology, Department of Neurosciences "B.B. Brodie", University of Cagliari.[2]Center of Excellence "Neurobiology of Dependence", University of Cagliari. [3]SER.T, A.S.L. n. 8, Cagliari.

23. MK-801 NEUROTOXICITY: BEHAVIOURAL AND HISTOLOGICAL EFFECTS IN RODENTS.

Bianchessi[1] S., Pecorini S.[2], Vaccani A.[1], Sala M.[2], Parolaio D.[1] and Gori E.[3]
[1]Dept. of Structural and Functional Biology, Pharmacology Section and Center of Neuroscience, University of Insubria, Varese, Italy. [2]Dept. of Pharmacology, Chemioterapy and Toxicology, Faculty of Sciences, University of Milan, Italy. [3]Zardi-Gori Foundation, Via Pietro Cossa 1, Milano, Italy

24. IN VITRO PHARMACOLOGICAL PROFILE OF THE NOVEL DELTA/MU OPIOID RECEPTOR LIGAND H-Dmt-Tic-Gly-NH-CH_2-Ph (UFP-505).

[1]Vergura R,[1]Valenti E, [2]Balboni G, [3]Hebbes CP, [3]Lambert DG, [1]Regoli D, [2]Salvadori S, and [1]Calo' G.

[1]Dept. of Pharmacology, [2]Dept. of Pharmaceutical Science, University of Ferrara, Italy. [3]Dept. of Cardiovascular Sciences, Pharmacology Group, University of Leicester, UK.

25. [Nphe^1,Arg^{14},Lys^{15}]NOCICEPTIN-NH_2 BLOCKED THE EXPRESSION OF OPIOID TOLERANCE.

Pieretti S. and Di Giannuario A.

Department of Drug Research and Evaluation, Istituto Superiore di Sanità, V.le Regina Elena 299, 00161 Rome, Italy.

26. NOCICEPTIN REDUCED THE INHIBITION INDUCED BY [Nphe^1,Arg^{14},Lys^{15}]NOCICEPTIN-NH_2 ON THE EXPRESSION OF OPIOID TOLERANCE.

Di Giannuario A. and Pieretti S.

Department of Drug Research and Evaluation, Istituto Superiore di Sanità, V.le Regina Elena 299, 00161 Rome, Italy.

ORAL PRESENTATION

ACTIVATION OF MULTIPLE TRANSCRIPTION FACTORS IN THE BRAIN OF CANNABINOID SENSITIZED ANIMALS.

Viganò D., Rubino T., Realini N., Castiglioni C. and D. Parolaro
DBSF, University of Insubria and Center of Neuroscience, via A. da Giussano 10, 21052 Busto Arsizio (VA), Italy

The present study tried to further elucidate the changes in brain functioning linked to stimulation of the CB1 receptor that might account for the expression of cannabinoid sensitization. Behavioural sensitization is an accepted model of neural plasticity within which drug-induced changes in complex behavior can be linked to drug-induced changes in molecular processes. On this basis the present study set out to examine changes in cannabinoid-induced activation of ERK in different brain areas of naïve animals and animals sensitized to THC. Alterations in the responsiveness of this intracellular cascade might be important in the expression of cannabinoid behavioral sensitization, acting on a wide range of effectors across many subcellular compartments. Since a major role for ERK lies in the regulation of gene expression, we also surveyed the transcription factors most closely related to this pathway, specifically p-CREB, c-Fos and Fos B in different brain areas of rats acutely treated with Δ9-tetrahydrocannabinol (THC) and exposed to a sensitization paradigm. THC challenge acutely raised the level of pERK immunoreactivity in the caudate putamen and cerebellum but in sensitized animal the response was still present only in caudate putamen. CREB phosphorylated levels were not altered in either the acute or sensitized protocol, while Fos proteins appeared to be regulated differently depending on the treatment schedule. Both c-Fos and Fos B increased significantly only in sensitized animals and the increase was restricted to brain regions involved in learning and memory (hippocampus, caudate putamen and prefrontal cortex). These data suggest that the brain response to THC in pre-exposed animals is different from naive animals and activates distinct neural circuits that contribute to the various aspects of behavioral sensitization.

CONDITIONED PLACE PREFERENCE (CPP) INDUCED BY SALVINORIN A IN WISTAR RATS

Limonta V., Braida D., Pegorini S., Gori E. and Sala M.
Department of pharmacology, chemotherapy and Medical Toxicology, University of Milan, Via Vanvitelli 32, 20129 Milan Italy

INTRODUCTION: Salvinorin A, the main pharmacological active component of *Salvia divinorum* is the first naturally occurring kappa opioid receptor agonist (Sheffler and Roth 2003; Chavkin et al. 2004) — and non-nitrogenous ligand —for opioid receptors. Salvinorin A has no structural resemblance to any known opioid ligand and represents a new class of kappa opioid selective compounds. Since there have been reports of *Salvia divinorum* self-administration in young people due to its hallucinogenic effect, the aim of the present study was to investigate the potential abuse of salvinorin A using the Conditioned Place Preference (CPP) test in Wistar rats.
MATERIALS AND METHODS**:** Pre-conditioned male Wistar rats were given salvinorin A s.c. (0.05-160 μg /kg) and after 5 min confined, for 30 min, in the drug-paired compartment. On the following day, rats received vehicle and confined to the opposite side. After eight of these conditioning sessions, CPP was assessed by measuring the time spent in each compartment. The role of k opioid receptor was investigated pre-treating rats, 35 min before salvinorin A, with nor-binaltorphimine (5 mg/kg i.p.).
RESULTS: Salvinorin A produced a significant increase in the time spent in the drug-paired compartment on the postconditioning day, between 0.1 and 40 μg/kg, when compared with that in the preconditioning period. Highest doses(80-160 μg /kg) produced aversion. Pretreatment with nor-binaltorphimine antagonized the rewarding effects of salvinorin A.
CONCLUSIONS: Even if activation of k opioid receptors is reported to induce conditioned place aversion (Mori et al., 2002), Salvinorin A appears to produce, at low doses, its reinforcing effects through k opioid receptors.
REFERENCES:
Sheffler D.J. and Roth B.L.(2003) Trends Pharmacol. Sci. 24:107-109;
Chavkin C., Sud S., Jin W., Stewart J., Zjawiony J.K., Siebert D.J., Toth B.A., Hufeisen S.J., and Roth B.L.(2004) J. Pharmacol. Exp. Ther. 308:1197-203;
Mori T., Nomura M., Nagase H., Narita M., Suzuki T.(2002) Psychopharmacology 161:17-22.

SINGLE OR REPEATED COCAINE TREATMENT MODULATES FGF-2 EXPRESSION IN RAT DOPAMINERGIC REGION

Fumagalli F.[1], Di Pasquale L.[1], Leo D.[2], Racagni G.[1]., Perrone-Capano C.[2,3]. and Riva M.A.[1].
[1]Center of Neuropharmacology, Pharmacological Sciences Dept., University of Milan; [2]IGB, CNR, Via Pietro Castellino 111, 80131 Napoli; 3 Dept. Pharmacobiol, Univ Catanzaro "Magna Grecia", I-88021 Roccelletta di Borgia (CZ).

FGF-2 (basic Fibroblast Growth Factor) is a trophic factor critical for dopaminergic neurons. We have recently shown that striatal FGF-2 expression is regulated by the dopaminergic system. Since dopamine is a crucial mediator of the action of cocaine, we hypothesized that alterations in FGF-2 expression may contribute to the adaptive mechanisms taking place in dopaminergic areas following cocaine administration. Since changes in neuronal responsiveness produced by cocaine are directly related to the dopaminergic transmission that originates in the ventral tegmental area, we decided to evaluate the expression in the midbrain of Nurr1, a critical dopaminergic marker. By using the highly sensitive RNase Protection Assay and semiquantitative Polymerase Chain Reaction techniques, we analyzed the expression of FGF-2 and Nurr1 in the striatum and midbrain of adult rats exposed to single or repeated injections (2 weeks) of cocaine at the dose of 5 mg/kg ip./daily. mRNA levels were measured 2 hours after the acute injection as well as 2 or 72 hours after the last injection of the chronic treatment. In striatum, single cocaine injection produced a 37% increase in FGF-2 expression ($p<0.01$, one way ANOVA) 2 hours after treatment whereas, after repeated treatment, FGF-2 expression was increased at both time points investigated (+38% and 24%, respectively) ($p<0.05$, one way ANOVA). In prefrontal cortex, the trophic factor expression was elevated by 56% ($p<0.01$, one way ANOVA) after single administration whereas repeated cocaine injections produced a 35% increase ($p<0.01$, one way ANOVA) 72 hours the last injection. In hippocampus and frontal cortex, no effects were observed after single injection whereas, in both regions, the repeated cocaine paradigm elicited a 19% and 36% increase, respectively ($p<0.05$, one way ANOVA). Interestingly, prolonged cocaine treatment significantly reduced Nurr1 expression in the midbrain both at 2 hours and 72 hours after the last injection (-50%) with no effects after a single administration. These results suggest that repeated cocaine treatment produces a finely tuned regulation of important determinants of dopaminergic neurotransmission that could represent, at least in part, the molecular basis of functional as well as behavioral alterations associated with abuse of cocaine.

METHYLPHENIDATE ADMINISTRATION TO ADOLESCENT RATS DETERMINES SHORT- AND LONG-TERM CHANGES ON REWARD-RELATED BEHAVIOR AND STRIATAL GENE EXPRESSION.

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Administration of methylphenidate (MPH, Ritalin®) to children with attention deficit hyperactivity disorder (ADHD) is an elective therapy but raises concerns for public health, due to possible persistent neurobehavioral alterations. Wistar adolescent rats (30- to 46-day-old) were administered MPH or saline, and tested for reward-related behaviors. Striata, a target of psychostimulants, were collected both at the end of the MPH treatment and at adulthood. To identify the molecular changes elicited by chronic MPH administration, we performed gene profiling of striatal transcripts by genome-wide microarray technique using Affymetrix RAE230A rat genome GeneChips. More than 700 genes were upregulated in MPH-treated rats (fold-change > 1.5). RT-PCR validations showed that four members of the postsynaptic-density (PSD) family, controlling plasticity of synaptic transmission, and five neurotransmitter receptors were up-regulated in the adolescent striatum after chronic MPH administration. Among PSD genes, *Homer1b*, *Shank2*, *MPP3* and *Dlgh2* transcripts were upregulated during adolescence. Interestingly, only genes for the kainate 2 subunit of ionotropic glutamate receptor (*Grik2,* also known as *KA2*/*GluR6*) and the 5-hydroxytryptamine (5-HT, serotonin) receptor 7 (*Htr7*) [but not *GABAA* subunits *β3* and *γ1* and adrenergic receptor (*Adr*) α1*b*] were still up-regulated at adulthood. CREB and *Homer 1a* transcrips were modulated only as a long-term effect. The effects were highly specific, since other genes belonging to the same families did not vary in the striatum, nor in the cerebellum.
Our data indicate short-term changes in neural plasticity, suggested by modulation of expression of key genes, and functional changes in reward-related striatal circuits. These modifications might in turn trigger enduring changes responsible for the adult neurobehavioral profile, i.e. altered processing of incentive values and persistence of some adolescent features.

IN VIVO EVIDENCE THAT LOCAL INJECTION OF SB-277011A INCREASES CELL FIRING IN THE VENTRAL TEGMENTAL AREA (VTA)

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Mesolimbic dopamine (DA) neurotransmission plays a central role in the neurobiology of relapse and drug-seeking behaviour. Among all DA receptor subtypes, the DA D3 receptor is highly expressed in brain reward-related regions, such as the ventral tegmental area (VTA), nucleus accumbens, and amygdala. Recent studies have shown that oral systemic administration of SB-277011A, a brain penetrant and selective DA receptor antagonist with 100-fold selectivity for the D3 over the D2 receptor *in vivo* (2), can alter the spontaneous activity of DA neurons in the VTA (3). However, in the same study the intravenous administration of SB-277011A failed to produce any significant effect on cell firing in the VTA. Such a discrepancy was related to different timings in the experimental procedures and/or to a putative metabolite of SB-277011A, which could be responsible for the alteration of DA neuronal activity (3). In an attempt to assess this possibility we have monitored DA neuronal spikes in the VTA in four groups of animals as described in previous studies (4). Following a 30-min control period, the first group was treated systemically (intraperitoneally, i.p.) with vehicle (water, 2ml/kg). The second group received an acute dose of SB-277011A (10 mg/kg i.p.) reported to be active on VTA cell firing (3). Finally, the third and fourth groups received a local injection of 1µl vehicle or 2.5µg/µl of SB-277011A just above the VTA. Data indicated that peripheral or local injection of vehicle was not influencing significantly pre- injection control values. Peripheral treatment with SB-277011A increased VTA cell firing up to 175±20% of control values within 10-15min. Local treatment with SB-277011A also resulted in a significant, larger increase of cell firing up to approximately 290±50% of controls within 5 min after treatment. These results show that local injection of SB-277011A into the VTA induced a more rapid and higher increase of neuronal activity than systemic treatment. These findings further strengthen the key role of DA D3 receptors in modulating mesolimbic DA activity.

1 Schwartz J.C., Levesque D., Martres M.P. and Sokoloff P. (1993) Clin Neuropharmacol. 16: 295-314;
2 Reavill C., Taylor S.G., Wood M.D. and Hagan J.J. (2000) J Pharmacol Exp. Ther. 294: 1154-1165;
3 Ashby Jr. C.R., Minabe Y., Stemp G. and Middlemiss D.N. (2000) J Pharmacol Exp. Ther. 294: 1166-1174;
4 Bunney B.S., Walters J.R., Roth R.H. and Aghajanian G.K.(1973) J Pharmacol Exp. Ther. 174: 560-571;

PRESYNAPTIC NICOTINIC RECEPTOR SUBTYPES MODULATING NEUROTRANSMITTER RELEASE: EFFECTS OF CHRONIC TREATMENT WITH NICOTINE.

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The objective of this study was to investigate the effects of chronic administration of (-)nicotine on function of the release-regulating presynaptic nicotinic receptors (nAChRs).
Adult male rats were anesthetized with chloral hydrate. (-)Nicotine bitartrate was dissolved with saline and the pH was adjusted to 7.4. Mini-pumps (model 2ML2; Alza Co., Palo Alto, CA) prefilled with either (-)nicotine bitartrate solution or saline were implanted subcutaneously to deliver 1mg/kg/hr of (-)nicotine bitartrate (8-10 mg/Kg/day of free base) for ten days. Crude hippocampal, striatal and accumbens synaptosomes were prepared as previously described (1).
In hippocampal synaptosomes, prelabeled with [3H]noradrenaline ([3H]NA), the nicotine-evoked overflow of [3H]NA was higher in nicotine-treated than in vehicle-treated rats.(2.9660.18% versus 2.3860.28% $P<0.05$). In striatal synaptosomes, prelabelled with [3H]dopamine ([3H]DA), chronic nicotine did not modify the releasing effect of nicotine.(0.6860.08% versus 0.7460.07%) No significant change could be observed in experiments with n. accumbens synaptosomes prelabeled with [3H]DA. The nicotine evoked of [3H]acetylcholine release was almost abolished in synaptosomes from treated animals, suggesting downregulation of nicotinic autoreceptors.(0.0560.02% versus 0.4560.05% $P<0.01$). In hippocampal synaptosomes prelabeled with [3H]D-aspartate ([3H]D-ASP), the releasing effect of epibatidine following chronic nicotine did not differ from that in controls. (nicotine treated : KCl(15mM) = 1.2660.08%; KCl(15mM)+Epibatidine(1μM) = 1.6260.08% ;vehicle treated : KCl(15mM) = 1.3060.12%; = 1.6260.08% KCl(15mM)+Epibatidine(1μM) = 1.7260.12%).
In conclusion the results show that chronic nicotine affects differentially the function of release-regulating nAChR subtypes.
This work was supported by a MIUR Network grant.
(1) Raiteri, L., Raiteri, M., (2000). Synaptosomes still viable after 25 years of superfusion. Neurochem. Res. 25, 1265-1274.

SERUM PROTEOMIC ANALYSIS DURING NICOTINE SELF-ADMINISTRATION, WITHDRAWAL, EXTINCTION AND RELAPSE IN RATS

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Nicotine dependence is known to induce long-term neural adaptations in the brain. The purpose of this study was to verify whether specific proteomics profiles related to nicotine dependence states could be identified in a peripheral tissue. Rats were trained to learn nicotine self-administration (n=6; 0.03 mg/kg/infusion) and serum was taken from the same animals at 6 time-points in distinct phases related to nicotine dependence (N, Naïve, control samples; P, Priming, was the 1st self-administration session; S, Self-administration, animals had been self-administering nicotine for 10 days; W, Withdrawal, access to nicotine was removed; E, Extinction, was the 4th extinction session; R, Relapse, nicotine was available again). Serum proteins were analysed by 2D electrophoresis, focussing them on immobilised 3-10 pH gradient strips followed by SDS-polyacrylamide gels and 72 maps were obtained. Image analysis was performed by PDQuest software (Bio-Rad). The spot volume values were submitted to statistical analyses: ANOVA F-test and a multivariate approach were performed to reduce the dataset complexity. In comparisons: N vs. S; S vs. W; E vs. R; S vs. R; and S vs. E a clear separation between the 2 compared groups could be observed by Principal Component Analysis, thus suggesting that each dependence state correlates with a protein expression pattern in serum. To single out which selections of proteins could better represent the contrasts, partial least squares-discriminant analysis was adopted to rank proteins by the contribution to the overall separation. The spots were identified by comparison with a published rat serum standard map (http://linux.farma.unimi.it/RSPSG/2D/index.html) and further analysed with a repeated-measure ANOVA test. Among them, C reactive protein and hemopexin displayed a significant reduction after nicotine administration, which was maintained throughout the administration paradigm. Two hemopexin isoforms and another unidentified protein were decreased in the S state. Thiostatin was increased in the E phase. This study showed that specific protein patterns related to the nicotine dependence states exist in peripheral tissues. Further development of this approach may provide robust diagnostic methods to assess dependence states of drug-taking individuals.

ACUTE AND REPEATED ADMINISTRATIONS OF MORPHINE AND PARACETAMOL: DIFFERENT EFFECTS ON NOCICEPTION, 5-HT2 AND μ- RECEPTORS IN THE RAT BRAIN

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It is well known that chronic administration of morphine induces tolerance to some behavioral effects, including analgesia in animals and humans. Since the therapeutic activity of some non-opioid analgesic drugs can induce tolerance, the first aim of the present work was to evaluate whether repeated administrations of paracetamol caused the phenomenon of tolerance to its antinociceptive effect in the hot plate test. We previously observed an increase in serotonin concentration in the cerebral cortex and in the pons of the rat accompanied by a decrease in the number of 5-HT2 receptors after acute paracetamol administration; also morphine has been proposed to exert its effect through serotonin release in some brain areas of the rat (1). Tolerance to the antinociceptive effect of morphine seems to depend on μ opioid receptors and with a lesser extent to κ-receptors. The second purpose was to evaluate the possible changes provoked by repeated administrations of morphine or paracetamol on the characteristics of μ , κ and 5 HT2 receptors in the frontal cortex of the rats treated with the same experimental schedule. Male rats were injected for 1 or 7 days (twice daily) with morphine (5 and 8 mg/kg, s.c.) or paracetamol (400 mg/kg, i.p.) to investigate the time course of the antinociceptive effect, evaluated by means of the hot-plate test. Either morphine or paracetamol induced an antinociceptive effect after acute administration (day 1) but only paracetamol maintained its effect for seven days while morphine did not. The number of μ opioid receptors in the frontal cortex was decreased at day 1, 3, and 7 in a similar percentage after paracetamol administration, while morphine produced a progressive decrease in respect to controls at the same day. The κ opioid receptors were unaffected by both paracetamol and morphine at any time of treatment. The 5-HT2 receptor number significantly decreased after paracetamol administration at day 1, 3, and 7; morphine behaved like paracetamol since it diminished the number of 5-HT2 binding sites in the same way. These data suggest that the opioidergic and serotonergic systems are involved, in different ways, in the induction and maintenance of antinociception after paracetamol or morphine treatment.
(1) Taylor B.K., Basbaum A.L. (2003) J. Neurochem. 86:1129-1141

ETHANOL PROMOTES STEROIDOGENESIS IN ISOLATED HIPPOCAMPUS: EFFECTS ON GABAR FUNCTION IN NORMAL AND ADX-CX RATS

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Recent data have suggested that certain behavioral and electrophysiological actions of EtOH may be mediated by an increase in brain concentrations of neuroactive steroids that results from stimulation of the HPA axis (1,2). Neuroactive steroids such as 3α,5α-THP are, in fact, potent and efficacious endogenous positive modulators of GABAA receptor function (3,4). Because neurosteroids can be synthesized *de novo* in the brain, we have investigated whether EtOH might affect both neurosteroid synthesis and GABAA receptor function in isolated rat hippocampal tissue. The concentrations of 3α,5α-THP in rat hippocampal minces after exposure to EtOH were assessed by RIA. The patch-clamp technique was applied to assess the effects of EtOH on GABAA receptor function in CA1 pyramidal neurons present in hippocampal slices. Bath application of EtOH (25-150 mM) increased in a time- and concentration-dependent manner the concentration of 3α,5α-THP as well as the amplitude and decay time constant of GABAA receptor-mediated IPSCs recorded from CA1 pyramidal neurons in isolated rat hippocampal slices. These effects were shared by GHB, CB34 (PBR agonist), and progesterone, all of which are known to increase the formation of neuroactive steroids in plasma and brain. The action of EtOH on GABAA receptor-mediated IPSCs is biphasic, consisting of a rapid, direct effect on GABAA receptors, and an indirect effect, which appears to be mediated by neurosteroids as it was prevented by the 5α-reductase inhibitor finasteride. To evaluate the contribution of steroid precursors originating from peripheral organs, the effects of EtOH, were also tested in adrenalectomized/castrated (ADX-CX) rats, where the amount of peripheral neuractive steroids is strongly reduced. In hippocampal slices from ADX-CX rats, both EtOH and GHB were still able to increase the concentrations of 3α,5α-THP and to modulate GABAA receptor-mediated mIPSCs at levels comparable to those in sham-operated animals. These observations suggest that EtOH and GHB may modulate GABAA receptor function through an increase in *de novo* neurosteroid synthesis in the brain that is independent of the HPA axis.

1 Barbaccia et al., Eur J Pharmacol 384:R1-2, (1999). 2 Morrow et al., Brain Res Rev 37:98-109, (2001). 3 Lambert et al., Brain Res Rev 37:68-80, (2001). 4 Sanna et al., J Neurosc., July 21, • 24(29):6521– 6530- (2004)

FORCED INTERMITTENT EXPOSURE (FIE) TO ETHANOL VAPOR AND WITHDRAWAL: A NOVEL ANIMAL MODEL FOR ALCOHOLISM

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Modelling the progression from low to high alcohol consumption has proven difficult in genetically non-selected laboratory rats. Exposure to ethanol vapor has been reported to transiently increase ethanol consumption, but this might be related to acute withdrawal. Here, we used a model based on repeated cycles of alcohol vapor exposure alternating with periods of mild withdrawal, and examined its long term consequences for ethanol intake and brain gene expression.
When three weeks of recovery were allowed to eliminate effects of acute withdrawal, exposed rats consumed markedly increased amounts of alcohol in a two-bottle free choice paradigm. This elevated alcohol consumption was observed up to a total time of 6 weeks following completion of the vapor exposure procedure. Acamprosate, which is clinically effective to reduce alcohol consumption in humans, fully counteracted this increase, without affecting alcohol intake in non-exposed subjects.
Presumably, long-term changes in gene expression patterns encode (mal)adaptive changes in the brain underlying the progression from low to high alcohol intake. Expression profiles were therefore compared in mRNA pools from different brain regions of exposed and control rats using oligonucleotide-based DNA-microarrays. Several transcripts were identified as being differentially expressed.
The exposure paradigm appears to be adequate to induce long-term changes in alcohol consumption of laboratory rats. It may serve as a model both to screen for suitable drug targets through gene expression profiling, and to validate such targets.

NOCICEPTIN/ORPHANIN FQ SYSTEM: BEHAVIORAL AND MOLECULAR DIFFERENCES BETWEEN MARCHIGIAN SARDINIAN PREFERRING RATS (msP) AND WISTAR RATS.

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Nociceptin/orphanin FQ (N/OFQ), the endogenous ligand of the opioid-like receptor NOP, reduces ethanol intake in genetically selected Marchigian Sardinian (msP) rats after cronic (7 days) treatment. The present study, was designed to evaluate the effect of this opioid peptide on ethanol self-administration in Wistar rats compared to msP animals. In addition, brain *in situ* hybridization studies for the NOP receptor and the N/OFQ peptide was performed in the two rat lines. Rats trained on a FR1 schedule of reinforcement to operantly self-administer 10% ethanol in 30 min daily sessions, received subcronic (6 days) ICV treatment with 0.5, 1.0 or 2.0 mg/rat of N/OFQ or its vehicle. For the in situ study two groups of ethanol naive msP and Wistar rats were sacrified, brain were collected and sectioned at the cryostat. Brain tissue sections (10mm) were hybridized using Nop (264 bp) and N/OFQ (260bp) riboprobes. Sense and antisense probes were labeled with S35-UTP.
Results showed that ICV treatment with N/OFQ (0.0, 0.5 or 1.0 mg/rat) markdly reduced ethanol self-administration in msP rats [$F(2,24)=7.30$; $p<0.01$)]. In contrast, in Wistar rats treatment with (0.0, 1.0 or 2.0 mg/rat) of N/OFQ was unable to control ethanol consumption [$F(2,27)=0.06$; NS]. In situ hybridization study demonstrated that, compared to Wistars, in msP rats there are significantly higher levels of NOP mRNA in the frontal cortex ($p<0.05$), in the central and basolateral amygdala ($p<0.01$) and in the CA3 region of the hippocampus ($p<0.05$). In msP rats higher N/OFQ mRNA expression levels were also found in the cingulate and frontal cortex ($p<0.05$), in the central amygdala ($p<0.01$), and in the bed nucleus of the stria terminalis ($p<0.01$). In conclusion, N/OFQ inhibits ethanol self-administration in msP but not in Wistar rats. Hibridization data underline a different expression levels of mRNAs encoding for the NOP receptor and the N/OFQ peptide in diffrents brain areas of msp rats compared to Wistras. This may explain, at least in part, the different response to exogenously administered N/OFQ observed in the two rat lines.
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ETHANOL-CRF-NOCICEPTIN INTERACTIONS AT GABAERGIC SYNAPSE IN RAT CENTRAL AMYGDALA

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Behavioral studies show that the GABAergic system in the central nucleus of the amygdala (CeA) plays a major role in the reinforcing effects of ethanol and in the anxiogenic response to ethanol withdrawal. Corticotrophin releasing factor (CRF) and nociceptin/orphanin FQ (N/OFQ) within the CeA are also implicated in regulating voluntary ethanol consumption and in the anxiogenic response to ethanol withdrawal. Using an electrophysiological approach, we have shown that 44 mM ethanol increases $GABA_A$ receptor-mediated inhibitory currents (IPSCs) in CeA neurons from both naïve and chronic ethanol treated (CET) rats, suggesting lack of tolerance for the acute effect of ethanol. Here we compared the effects of CRF and N/OFQ on IPSCs in CeA neurons from naïve and CET rats. We found that 200 nM CRF, like ethanol, significantly enhanced evoked IPSC amplitudes (to 140%, n = 9) in CeA of naïve rats, in part via presynaptic CRF1 receptors. N/OFQ (500 nM) moderately decreased IPSC amplitudes and prevented the ethanol- and CRF-induced increase of IPSCs. N/OFQ at least in part acts presynaptically because it decreases mIPSC frequencies. Interestingly, in CeA of CET rats the ability of CRF to increase IPSCs was enhanced (to 165%, n = 9) compared to naïve rats. Similarly, the N/OFQ-induced inhibition of IPSCs was increased in neurons of CET rats, indicating an enhanced sensitivity to N/OFQ. Our data support the hypothesis that chronic ethanol enhances the sensitivity of GABAergic systems in CeA to these two neuropeptides.
Supported by grants from the NIAAA (AA013517-INIA Project, AA013498-INIA Project and AA-06420) and NIDA DA13658.

ROLE OF THE NOCICEPTIN/ORPHANIN FQ – NOP RECEPTOR SYSTEM IN MORPHINE TOLERANCE: PHARMACOLOGICAL AND KNOCKOUT STUDIES

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Nociceptin/orphanin FQ (N/OFQ) via N/OFQ peptide receptor (NOP) activation reduces several biological effects of morphine thus behaving as an anti-opioid system. Here we report on the involvement of the N/OFQ – NOP receptor system in tolerance to morphine analgesia. Morphine tolerance was assessed using the mouse tail withdrawal as analgesiometric assay and the role of the N/OFQ – NOP receptor system was evaluated with mice knockout for the NOP receptor (NOP-/-) and with J-113397, the non peptide NOP selective antagonist. **Methods**. Morphine analgesic effects were measured in the tail withdrawal assay, cut off time of 10 s, water 53 °C. Withdrawal latencies were measured before and after 5, 15, 30 and 60 min from the morphine injection. Row data were converted in and expressed as area under the curve for the period 5-60 min. Mice were treated with morphine s.c. twice a day for 4 days according to the following schedule: day I: a.m. 5 mg/kg, p.m. 10 mg/kg; day II: a.m. and p.m. 30 mg/kg; day III: a.m. and p.m. 50 mg/kg; day IV: a.m. and p.m. 100 mg/kg. On day V, mice were challenged with 5 mg/kg morphine. **Results.** Mice chronically treated with morphine displayed tolerance to the analgesic effect of 5 mg/kg morphine (day I 1924 ± 257; day V 562 ± 95). The administration of J-113397 10 mg/kg 15 min before the last morphine injection, produced a statistically significant reduction of morphine tolerance (day I 2130 ± 378; day V 1317 ± 186). However, J-113397 also potentiated the effect of 5 mg/kg morphine in naive nice, although this latter effect did not reach the level of significance (control 1581 ± 340; J-113397 2877 ± 491). NOP+/+ and NOP-/- mice were similarly sensitive to both the acute analgesic effect of morphine and to the development of morphine tolerance. **Conclusions**. Results obtained with the NOP antagonist J-113397 indicate that the endogenous N/OFQergic signalling contrasts morphine analgesia and that this action is more pronounced after chronic treatment with the alkaloid. These findings were not confirmed by knockout studies: compensatory mechanisms, i.e. higher activities of other anti-opioid systems, may probably account for these differences.

CHRONIC INFUSION WITH BUPRENORPHINE DECREASES NOP RECEPTOR DENSITY IN RAT BRAIN.

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Buprenorphine is a potent opioid analgesic showing high affinity to μ, δ and κ opioid receptor and a slow receptor dissociation, used to treat moderate to severe pain. More recently it has also been proposed in the treatment of opioid dependence. The ability of buprenorphine to bind and activate the nociceptin receptor (NOP), has been reported (1). The endogenous ligand for this receptor, that is nociceptin/orphanin FQ (N/OFQ), is known to exert an anti-opioid effect, at supraspinal level (2). This activation of an anti-opioid system by buprenorphine, has been recently proposed (3) as the responsible for its bell-shaped dose-response antinociceptive curves.
Here we evaluated the effects of a chronic infusion of buprenorphine on NOP receptor density in some rat cerebral areas involved in nociceptive transmission.
Male Sprague–Dawley rats (250 ± 10 g) were treated with buprenorphine hydrochloride (0.3 mg/kg/die) or vehicle (controls) by means of subcutaneously implanted osmotic minipumps releasing the opiate at the rate of 3.125 μg/h. After seven days, brain areas were dissected out and NOP receptor density was determined in the thalamus, hippocampus and frontal cortex by homologous competition binding studies, using [leucyl-3H]-N/OFQ as radioligand (1 nM, chosen on the basis of saturation studies) and cold N/OFQ (10 pM-10μM) as displacer.
In all three investigated areas, the prolonged s.c. infusion with buprenorphine caused significant changes in NOP receptor density and the following Bmax values were determined. Thalamus: 164.0 ± 9.8 vs 256.7 ± 20.5 fmol/mg protein in treated and control rats, respectively ($p < 0.05$); hippocampus: 159.6 ± 15.6 vs 377.0 ± 25.7 ($p < 0.01$); frontal cortex: 112.1 ± 14.9 vs 159.5 ± 6.8 ($p < 0.05$).
These results indicate that neurochemical changes of the N/OFQ-NOP system occur after the prolonged administration of buprenorphine. The decrease of NOP density observed in the investigated areas could reduce the functional antagonism exerted by the NOP activation on opiate analgesia, potentially leading to positive effects of continuous infusion protocols in the clinical use of the opiate.

1) Huang P., Kehner G.B, Cowan A. et al. (2001) J. Pharmacol. Exp. Ther. 297: 688-695.
2) Mogil J.S., Pasternak G.W. (2001) Pharmacol. Rev. 53: 381-415.
3) Lutfy K., Eitan S., Bryant C.D., Yang C.Y. et al. (2003) J. Neurosci. 23: 10331-10337.

POSTER PRESENTATIONS

BEHAVIORAL AND NEUROCHEMICAL MODIFICATIONS IN MORPHINE SENSITIZED RATS 10-20 DAYS AND 6 MONTHS AFTER THE LAST DRUG ADMINISTRATION

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Morphine sensitized rats present intense stereotypies upon challenge with a low dose of morphine and are resistant to the behavioral sequelae of unavoidable stress exposure; both these effects are selectively dependent on DA D1 receptor activation. Moreover, they show an increase in the functioning and expression of Gs, a decrease in Gi proteins, and an increase in basal adenylyl cyclase activity in striatal areas. A major target protein for the cAMP/cAMP-dependent protein kinase (PKA) pathway in the striatal areas is DA and cAMP-regulated phosphoprotein (Mr 32 kDa) (DARPP-32), a protein that can act either as a phosphatase inhibitor or as a kinase inhibitor, depending on its phosphorylation state. Repeated administrations of many drugs of abuse induce the accumulation in the ventral and dorsal striatum of ΔFosB, a complex of modified isoforms of Fos family proteins that persist in the neurons for several weeks because of their long half-lives. We studied these parameters in morphine sensitized rats 20 days and 6 months after the last morphine administration. Morphine sensitization at 20 days was associated with increased expression of ΔFosB, no modification in the expression of Cdk5, and no modification in the basal phosphorylation pattern of DARPP-32. An acute morphine challenge in morphine sensitized rats increased the levels of phospho-Thr75 DARPP-32 and decreased those of phospho-Thr-34 DARPP-32 in a time dependent manner, both in the dorsal and ventral striatum. The acute exposure to unavoidable stress also transiently increased phospho-Thr75 DARPP-32 and decreased phospho-Thr-34 DARPP-32 levels specifically in morphine sensitized rats. Interestingly, the acute administration of SCH 23390 before stress exposure reverted the condition of stress resistance and prevented the modifications in DARPP-32 phosphorylation pattern; whereas the administration of naloxone had no effect. Six months after the sensitization procedure, morphine sensitized rats still presented DA D1 receptor-dependent stereotypies after a morphine challenge and were completely resistant to unavoidable stress exposure. However, the neurochemical parameters examined were indistinguishable from those of 6-month old control rats.

THE EXPRESSION OF COX-2 AND iNOS ENZYMES BY MORPHINE WITHDRAWAL.

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In a recent paper, we have shown that both COX1 and COX2 are involved in the expression of opioid withdrawal since both tolmetin (COX1 inhibitor) and meloxicam (COX2 inhibitor) are able to reduce morphine withdrawal. Furthermore, we noted that meloxicam compared to tolmetin was more active in inhibiting morphine withdrawal suggesting that prostaglandins (PGs) involved in the development of opioid withdrawal may be mainly produced by COX2 rather than COX1. The aim of the present work was to investigate the possible role of COX2 and iNOS in the development of morphine withdrawal considering that Nitric Oxide stimulates PGs biosynthesis through a direct interaction with COX enzymes. Therefore, the effect of morphine on the expression on COX-2 and iNOS was considered by evaluating:

1. the effect of morphine on COX-2 protein expression in LPS-stimulated J774 macrophages. Treatment of J774 macrophages with LPS caused an accumulation of PGs. The addition of morphine to the cells 30 min before LPS challenge increased significantly PGs production. The COX-2 immunofluorescence study indicated that the control cells showed only background staining, cell stimulated with LPS alone revealed a diffuse accumulation of COX-2 immunostaining in the cytoplasm: this accumulation of COX-2 immunostaining increase significantly in cells stimulated with LPS+Morphine. This effect was reverted by naloxone.
2. the effect of morphine on NO production by LPS-stimulated J774 macrophages. Treatment of J774 macrophages with LPS caused an significant increase of NO Morphine added to the cells 0.5 h before activation with LPS, further increased NO production. This effect was reverted by naloxone. Morphine was not able to increase NO production when added after LPS challenge.

The present paper provides a strong evidence that both PGs and NO are involved in the development of morphine withdrawal further indicating that during morphine withdrawal the opioid induces the expression of the COX-2 and iNOS enzymes

CHRONIC BACLOFEN REVERSES, BUT DOES NOT PREVENT, MORPHINE – INDUCED MOTOR SENSITISATION

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Rationale: The mesocorticolimbic dopaminergic pathway and its modulation by GABAergic system are thought to play a fundamental role in the mechanisms underlying drug addiction. Thus many preclinical and clinical studies have evaluated the potential of $GABA_B$ agonists as a pharmacotherapy for substance abuse (1).
Objectives: Baclofen co-administration has been reported to block the acquisition and expression of opiate-induced motor sensitisation (2). In this line the present study was undertaken to find out if chronic baclofen could reverse (Experiment 1) and/or prevent (Experiment 2) morphine sensitisation in rats.
Methods: Four groups of male Sprague-Dawley rats have been i.p. treated first with 10 mg/kg morphine (G1-G2) or saline (G3-G4) daily for 10 days, second with 2 mg/kg baclofen (G1-G3) or saline (G2-G4) daily for further 10 days; finally all the animals were tested with 10 mg/kg morphine, 3 and 30 days after ceasing the chronic treatment (Exp.1).
Subsequently all the rats received 10 mg/kg morphine (G1-G3) or saline (G2-G4) i.p. daily for 10 days and were tested with 10 mg/kg morphine 10 days after ceasing the chronic treatment, in order to evaluate if a previous repeated treatment with baclofen could prevent the development of sensitisation in "morphine-naive" (G3) and/or "morphine experienced" (G1) rats (Exp.2).
Results: As expected, morphine locomotor effect underwent a strong sensitisation in control animals; chronic baclofen, at a dose that did not affect motor activity by itself, completely reversed morphine sensitisation and its effect was long-lasting (Exp.1).
However chronic baclofen did not prevent the sensitisation to the locomotor stimulant effect of morphine either in "morphine-naive" or "morphine-experienced" rats (Exp. 2).
Conclusion: Sensitisation phenomena are postulated to contribute to compulsive drug taking, drug craving and relapse (3). In this line the present results indicate that $GABA_B$ agonists may be useful in the treatment, but not in the prevention of opiate addiction.

References:
(1) Cousins M.S., Roberts D.C.S., DE WIT H. (2002) Drug and Alcohol Dependence 65: 209-220;
(2) Kimberly A., Leite-Morris K.A., Fukudome E.Y., Shoeb M.H. and Kaplan G.B. (2004) J Pharmacol Exp Ther 308 (2): 667-678;
(3) Robinson T.E. and Berridge K.C. (2003) Annu. Rev. Psychol 54 : 25-53.

DIFFERENT IMPACT OF CHRONIC TRAMADOL OR MORPHINE ON PRODYNORPHIN GENE EXPRESSION, IN RAT BRAIN AREAS.

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The mechanisms underlying the development of tolerance and dependence are still not completely understood. However, it is now accepted that they can be viewed as drug-induced neural plasticity involving either cellular (e.g. receptor function) or molecular (e.g. gene expression) changes (1). At the molecular level, the neurochemical mechanisms involved in the development of tolerance to, and dependence on, opiates include homologous regulation, affecting the endogenous opioid system, along with heterologous regulation involving other transmitter systems (2).
A low abuse liability is reported for tramadol (3), an analgesic drug centrally acting through either opioid or non opioid mechanisms. In this paper, we evaluated the effects of the repeated administration of different doses of tramadol on the opioid precursor prodynorphin biosynthesis, in comparison with morphine, in the rat central nervous system (CNS).
Male Sprague-Dawley rats, subdivided into four groups (n=10), were daily administered i.p. with saline (2 ml/kg), morphine (10 mg/kg) or different doses of tramadol (10, 20 and 80 mg/kg). On the first day, and thereafter on alternate days (i.e. on days 3, 5 and 7), the antinociceptive threshold was determined by means of the tail-flick. Two h after the last administration (day 7), rats were sacrificed, and the hypothalamus, hippocampus and striatum were dissected out and processed for Northern analysis.
Northern analysis showed that morphine and tramadol produced different effects. Morphine caused a down-regulation of prodynorphin mRNA levels in all investigated areas (hypothalamus, hippocampus and striatum) whereas tramadol did not cause any significant change in the striatum, and did not decrease prodynorphin biosynthesis in the hypothalamus and in the hippocampus, at non-toxic doses (10 and 20 mg/kg). The highest dose of tramadol (80 mg/kg) decreased prodynorphin mRNA levels in the hypothalamus and the hippocampus but not in the striatum. These data give some information on tramadol effects at molecular level in the CNS. They indicate that the alterations of prodynorphin gene expression caused by tramadol and morphine show a different pattern that may be related to the different abuse potential of the two analgesic drugs.

1) Nestler E.J. et al., Neuron, 11, 995-1006, 1993.
2) Koob J:F, Le Moal M., Science, 278, 52-58, 1997
3) Cicero T.J. et al., Drug Alcohol Depend., 57, 7-22, 1999

PRENATAL EXPOSURE TO CANNABINOID AGONIST AFFECTS MOLECULES INVOLVED IN INTRACELLULAR SIGNALING PATHWAYS

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It is well accepted that adverse life events occurring early in development may alter the correct program of brain maturation leading to an enhanced vulnerability to neuropsychiatric disorders. Some of these factors become very important during development either alone or in combination with genetic factors, increasing the vulnerability to adverse events later in time. Recently, Mereu and coworkers (Mereu et al., PNAS, 2003) have demonstrated that prenatal exposure to the CB1 receptor agonist WIN 55212-2 produces memory deficit in adulthood. This effect is associated to a reduced functionality of the glutamatergic system suggesting that cannabinoids exposure early in life have a strong impact on the function of mature brain. The aim of our study was to identify molecular changes produced by gestational exposure to WIN 55212-2, which might contribute to late disruption in synaptic plasticity and cognition. For this purpose, WIN 55212-2 was injected in dams from gestation day 5 to 20 (0,5 mg/kg x 2/die or Vehicle 0,1 ml/100g x2/die) and the levels of total and phosphorylated ERK1/2 and CaMKII, two signaling molecules important for cognition, were analysed. These measures were carried out in different cellular fractions by western blot analysis in adult rats that were exposed in utero either to the CB1 receptor agonist or to vehicle. We found that prenatal exposure to WIN 55212-2 significantly reduced the phosphorylated levels of ERK1 in hippocampal membrane and, to lesser extent, in the nuclear and in the cytosolic compartments, whereas no changes were observed in P-ERK2 or in total levels of both isoforms. The levels of P-ERK-1 were decreased by prenatal WIN 55212-2 treatment also in the nuclear fraction of prefrontal cortex. Furthermore a robust reduction of phospho-CaMKII was also found in the hippocampus of rats prenatally exposed to the CB1 agonist, with a concomitant decrease of the expression for the neurotrophin BDNF. Our data suggest that deficit in ERK1/2 and CAMKII pathways might contribute to cognitive and neuroplastic defects associated with the prenatal exposure to cannabinoids.

SELECTIVE INHIBITION OF REWARD-RELATED BEHAVIOURS BY THE CANNABINOID CB1 RECEPTOR ANTAGONIST SR-141716A IN RATS.

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The endocannabinoid system has been suggested to be involved in the regulation of ethanol consumption in rats and mice (1, 2). The present study investigated in rats the effect of SR-141716A, a selective CB1 receptor antagonist, on 10% ethanol self-administration, under fixed-ratio 1 (FR1) and progressive ratio (PR) schedule of reinforcement and on the reinstatement of extinguished ethanol-seeking behaviour induced either by stress or by cues. The selectivity of the effect SR-141716A was tested on 5% sucrose self-administration as well as on 2% NaCl self-administrration in sodium depleted rats. Wistar rats received an intraperitoneal injection of SR-141716A 30 min before the self-administration sessions. The results showed that treatment with SR-141716A (0.3-1.0-3.0 mg/kg) dose-dependently attenuated lever pressing for 10% (v/v) ethanol under both the FR1 [$F(3,8) = 10.245$; $p < 0.001$] and PR schedules of reinforcement [$F(3,7) = 6.397$; $p < 0.01$]. SR-141716 markedly inhibited also the reinstatement of extinguished alcohol-seeking behaviour elicited by presentation of cues predictive of drug availability [$F(3,8) = 6.664$; $p < 0.01$]. Conversely, neither 1.0 nor 3.0 mg/kg of the CB1 receptor antagonist prevented the reinstatement of alcohol-seeking induced by foot-shock stress ($p>0.05$, NS). Moreover, SR-141716A (0.3-1.0-3.0 mg/kg) decreased 5% sucrose self-administration ($p<0.01$), whereas lever responding for NaCl in sodium depleted animals was not influenced by the drug ($p>0.05$, NS). There results emphasize that endocannabinoid mechanisms play a major role in the control of ethanol self-administration and the conditioned reinstatement of ethanol-seeking behaviour. This effect may depend on an inhibition of reward-reinforced operant responding. In fact, the same doses of SR-141716 reduced also responding for sucrose but not for the non palatable 2% NaCl solution in animals motivated to take it by sodium depletion. The use of SR-141716 for the treatment of alcohol abuse may represent an interesting pharmacotherapeutic option.
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1) McMillan et al (1991) Drug and alcohol dependence 27:263- 2742.
2) Hungund BL, et al.(2003) J Neurochem. 84:698-704

NEUROPHARMACOLOGICAL EVALUATION OF *CANNABIS SATIVA* ESSENTIAL OIL.

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The first formal report of *Cannabis sativa* L. as a medicine appeared in China nearly 5000 years ago when it was recommended for malaria, constipation, rheumatic pains. It was not until the XIX century that *Cannabis* became a mainstream medicine in Britain as antispasmodic, antiemetic, and hypnotic. For these properties galenic preparations with *Cannabis* were present in most of the US and European pharmacies. In the first years of XX century, *Cannabis* began to be abused widely for its intoxicating effects and its medical use declined considerably.
The peculiarity of *Cannabis* is its smell. Its aroma does not originate from the terpenophenolic cannabinoids, but from the more volatile monoterpenes and sesquiterpenes. The aim of this work was to investigate a possible role of essential oil extracted from *C. sativa*, since no pharmacological data has been reported on the volatile oil, so it seemed interesting to evaluate its possible effect on central nervous system (CNS).
In order to verify its neuropharmacological properties the spontaneous motility using the activity cage test, the pentobarbital induced sleeping time, and the pentylenetetrazole induced seizures have been observed.
The *in vivo* results showed a depressing activity on CNS in our experimental condition; locomotory activity was significatively reduced (49%) by the oil administration even at very low doses (0.01 ml/Kg). The sleeping time was prolonged to twice its lenght. The severity of the seizures was mitigated in pretreated animals (0.1 ml/Kg and 0.05 ml/Kg). All unpretreated animals had an high mortality percentage, on the contrary all pretreated animals survived.
It is difficult, at the moment, to interpret exactly the mechanism of action of compounds present in the mixture of the volatile oil, even if the scientific literature indicates the efficacy of some monoterpenic principles, as linalool, eugenol or anethole.
On the other hand the presence of cannabinoid structures seemed not strictly necessary to obtain a neuropharmacological activity. These results may stimulate further investigations which will be useful to ascertain a possible involvement of monoterpenic structures.

EFFECT OF CANNABINOID CB1 RECEPTOR AGONISTS ON 5-HT EXTRACELLULAR LEVELS IN DIFFERENT RAT BRAIN AREAS

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The endogenous cannabinoid system has been suggested to be involved in the control of emotional states. Several studies showed that administration of CB1 receptor agonists induces modifications of the behavioural state causing anxiolytic or anxiogenic effects both in human and rats. Within the CNS the serotonergic system is considered one of the most relevant neurotransmitter systems involved in several psychiatric disorders and represents a successful therapeutic target for most forms of anxiety. Since experimental data have pointed out a behavioral and biochemical interaction between central serotonergic and cannabinoid systems, in this study we decided to evaluate the effect of acute cannabinoid CB1 receptor agonist (THC and WIN 55,212-2) administrations on extracellular 5-HT levels in the shell part of the nucleus accumbens, dorsal striatum and basolateral amygdala, by using the "in vivo" microdialysis technique in rats.

Intravenous administration of a low dose of either WIN 55,212-2 or THC (0.15 mg/kg) increased 5-HT extracellular levels in the rat nucleus accumbens and striatum with respect to basal values. On the contrary, a higher dose of WIN 55,212-2 (0.3 mg/kg) elicited a significant decrease of 5-HT release whereas THC at dose of 0.30 mg/kg did not. These effects were specifically reversed by the CB1 receptor antagonist Rimonabant (SR141716A 0.3 mg/kg ip) that did not modify 5-HT release *per se***.** The two doses of WIN 55,212-2 tested induced only a weak effect if any in the medial prefrontal cortex and basolateral amygdala respectively.

Our data demonstrate that administration of cannabinoid agonists differently modulates 5-HT release in the nucleus accumbens, striatum, medial prefrontal cortex and amygdala, suggesting a different involvement of the serotonergic neutransmission in the behavioural and emotional states induced by cannabinoids.

(1) Taylor B.K., Basbaum A.L. (2003) J. Neurochem. 86:1129-1141

EFFECTS OF A CANNABIS EXTRACT ON MOTOR ACTIVITY, ANXIETY AND NOCICEPTION IN THE RAT: INVOLVEMENT OF THE SEROTONERGIC SYSTEM

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Cannabis derivatives have been shown to produce many behavioral modifications in rodents as well as humans (1). A marked increase in the dose of Cannabis self-administration in drug abusers has recently described.

The first purpose of the present study was to investigate, in rats, the effect of doses ranging from moderate to high of a cannabis extract on some behavioral patterns known to be affected by cannabinoid consumption. Several results showed that serotonin is involved in the mechanisms of action of behaviors involving mood, emotions and pain modulation. Therefore the second purpose of our study was to assess whether the serotonergic system was involved in some of these behavioral changes.

Wistar adult male rats were administered per os with 25, 50 and 100 mg/kg of a standardized cannabis extract containing 10% ⊗9-tetra-hydrocannabinol (⊗9-THC) 30 min before the behavioral tests. Spontaneous coordinate activity, was recorded continuously for 20 min in a soundproof room. Antinociception was evaluated by means of the hot plate and von Frey filament tests. The level of anxiety was analysed using the elevated plus maze test. Immediately after hot plate test the rats were decapitated, frontal cortex removed and utilized for serotonin level and 5-HT2 receptor evaluation.

The number of movements was decreased in treated rats in a dose-dependent manner while the %MPE values were significantly increased dose-dependently as well. All the doses of the extract produced an anxiogenic-like effect indicated by the decrease in the time spent and in the number of entries in the open arms. Preliminary data concerning at the highest dose used indicate a parallel biochemical effect of the cannabis extract which increased the 5-HT levels and decreased the 5-HT2 receptor number in the frontal cortex.

The results match the hypothesis that 5-HT in the brain is involved either in nociception or in anxiety and that it might be modulated, as well as other neurotransmitters, by cannabinoids.

(1) Chaperon F., Thiebot M.H. (1999) Crit. Rev. Neurobiol. 13:243-281.

CROSS-TALK BETWEEN CB1 AND NOP RECEPTORS IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS

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We used neuroblastoma SH-SY5Y cells as a model to investigate the possible interaction between cannabinoid and nociceptin-NOP receptor systems. The neuropeptide nociceptin (also named as orphanin FQ, N/OFQ) is the endogenous ligand for the ORL-1 receptor, recently referred to as NOP. This receptor presents marked structural analogies with the three different opioid receptors, nevertheless it is not able to interact with the ligands for such receptors. The pharmacological characterization of this neuronal system allowed to suggest that nociceptin acts as a functional antagonist towards the endogenous opioid system.
Previous studies showed a functional modulation between the opioid and cannabinoid systems (1, 2) and evidence has been provided that the endogenous opioid system is involved in the regulation of several effects elicited by cannabinoids, such as analgesia, reward, immunological responses or anxiety-like behaviour (2).
Here we investigated the effects of delta9-THC exposure on NOP gene expression, receptor density and N/OFQ peptide levels in the human neuroblastoma SH-SY5Y cell line. Furthermore, CREB activation was measured by Western blot analysis. The cells were exposed to 50-200 nM delta9-THC for 24 hours. RT-PCR analysis showed a decrease of NOP receptor mRNA levels after delta9-THC exposure (97.5 ± 1.5%, 62.8 ± 0.9%, 51.0 ± 4.0, 49± 2% vs controls (= 100%,) for 50 nM, 100 nM, 150 nM and 200 nM delta9-THC exposure, respectively, $p<0.05$).
A dose-dependent decrease of NOP receptor Bmax was observed after exposure to delta9-THC (73±5%, 67±3.5% and 62±5% versus controls=100%, for the concentrations of 100, 150 and 200 nM , respectively, $p<0.05$). Exposure of SH-SY5Y to delta9-THC did not change the cell N/OFQ-ir content but decreased the medium N/OFQ-ir content (190.2 ±14.5, 114.4±9.6, 114±10.2, 112±13.4 fmol/mg protein vs 239.8 ±3.5 fmol/mg protein (controls) for the concentrations of 50, 100, 150 and 200 nM , respectively, $p<0.05$). Western blot analysis evidenced, for the first time, a dose dependent inhibition of CREB phosphorilation after cell exposure to delta9-THC (87.4, 74.5, 74.5 and 69.0 % vs controls 100%, for the concentrations of 100, 150 and 200 nM , respectively).
These findings show that delta9-THC is able to affect nociceptin/NOP system, providing evidence for the existence of interactions between nociceptin/NOP and cannabinoid systems. In addition these data suggest the involvement of CREB pathway in the cross-talk between CB1 and NOP receptors.
References
1. Braida D, Pozzi M, Parolaro D, Sala M. Eur J Pharmacol. Feb 16;413(2-3):227-344, 2001.
2. Cichewicz DL. Life Sci. 30;74(11):1317-24, 2004.

COCAINE AFFECTS NOP RECEPTOR GENE EXPRESSION IN SH-SY5Y CELLS

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The neuropeptide nociceptin (also named as orphanin FQ) is the endogenous ligand for the ORL-1 receptor, recently referred to as NOP. This receptor presents marked structural analogies with the three different opioid receptors, nevertheless it is not able to interact with the ligands for such receptors. The pharmacological characterization of this neuronal system allowed to suggest that nociceptin acts as a functional antagonist towards the endogenous opioid system.
Several studies have demonstrated that cocaine has profound effects on the endogenous opioid system (1) and recently it was observed that OFQ/N blocks cocaine-induced behavioral sensitization through activation of the ORL-1 receptor (2).
The aim of the present study was to investigate the possible change of nociceptin/NOP receptor system after cocaine exposure in SH-SY5Y cells. The cells were exposed to 1-50 μM cocaine for 24 hours. Cocaine significantly up-regulates NOP receptor gene expression both in undifferentiated and cells differentiated with retinoic acid. No changes were observed in NOP Bmax.
Exposure of SH-SY5Y to cocaine did not change the cell N/OFQ-ir content but increased the medium N/OFQ-ir content (633 ± 44 fmol/mg protein vs control (=325 ± 26 fmol/mg protein) for 50 μM exposure, *$p<0.05$, Dunnett's test).
These results indicate that cocaine is able to alter NOP receptor gene expression and the medium N/OFQ-ir content. This is the first evidence that, as well as cocaine administration is able to produce alterations in opioid receptor gene expression, it is also able to regulate NOP gene expression.
Our results are in agreement with other literature data (3) and suggest a role of the N/OFQ-NOP system in regulating responses to psychostimulant drugs and strengthen the hypothesis of a possible involvement of nociceptin and its receptor in the mechanisms activated by cocaine.
References:

1. Unterwald EM, Ann N Y Acad Sci. 937:74-92, 2001
2. Lufty K, Khaliq I, Carroll FI, Maidment NT. Psychopharmacology (Berl.) 164(2):168-76 2002
3. Narayanan S, Lutfy K, Maidment N. Behav Brain Res. 131(1-2):97-103 2002

SUBCHRONIC ADMINISTRATION OF COCAINE IN THE RAT INDUCES AN UP-REGULATION OF NOCICEPTIN RECEPTOR mRNA EXPRESSION IN THE CORE OF NUCLEUS ACCUMBENS

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Orphanin FQ/Nociceptin (OFQ/N) system has been shown to be involved in the regulation of emotional behaviours and it has been recently suggested to have a role in the modulation of mesolimbic dopaminergic neurotransmission. The aim of the present study was to investigate the mRNA expression of OFQ/N receptor (NOP) in the mesolimbic-dopaminergic system and in some limbic-related regions of the rat brain following acute or subchronic administration of cocaine. Adult Wistar rats (6/group) were exposed to either a single or a repeated (once daily for 5 days) admistration of cocaine (15 mg/Kg, i.p.); control rats were treated with saline. All the animals were sacrificed 1 hour and 30 minutes after the last administration. *In situ* hybridization experiments were then performed using a specific riboprobe for NOP receptor, generated from a cDNA fragment that spans 600 base pairs (101-700) of the rat sequence (accession number NM_031569). Subchronic treated rats presented a significant up-regulation of the NOP mRNA expression in a dopaminergic region, the core of nucleus accumbens ($p = 0.002$), as well as in some limbic regions, such as the cingulate cortex ($p = 0.004$), the septum ($p = 0.0085$), the CA1 ($p = 0.032$), CA2 ($p = 0.011$) and CA3 ($p = 0.011$) hippocampal regions. No statistically significant effects were seen in these regions after the acute treatment. No differences were seen in the other regions analyzed, such as medial prefrontal cortex, shell of the nucleus accumbens, caudate putamen, dentate gyrus of hippocampus, paraventricular nucleus of hypothalamus, central amygdala, dorsal raphe and locus coeruleus. The present results, namely the increase of NOP receptor mRNA expression levels after subchronic administration of cocaine, might be explained as a mechanism of negative feedback of the OFQ/N system, in view of the previous findings showing that OFQ/N administered centrally dose-dependently attenuates the motor stimulatory effect of cocaine. The up-regulation of NOP mRNA in the core of the nucleus accumbens, a brain region implicated in the rewarding action of cocaine and other drugs of abuse, provides further support to the hypothesized involvement of OFQ/N system in the mechanisms underlying drug dependence.

ROLE OF DIFFERENT NEUROTRANSMITTERS ON COCAINE-MEDIATED EFFECTS ON PRODYNORPHIN GENE EXPRESSION AND CREB ACTIVATION IN THE RAT BRAIN

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Cocaine binds to dopamine (DA), serotonin (5-HT) and norepinephrine (NE) transporters blocking the reuptake of these monoamines into presynaptic terminals. As previously reported, continuous infusion of cocaine for seven days or GBR 12909, a selective dopamine uptake inhibitor, produced a significant decrease in prodynorphin (PDYN) gene expression in the hypothalamus and increase in PDYN mRNA in the caudate putamen (CP). The effect of the selective serotonin uptake inhibitor fluoxetine was examined on PDYN gene expression and on CREB activation. Fluoxetine or vehicle was infused continuously for 7 days via osmotic minipumps into male rats. Northern blot analysis showed significant increases in PDYN gene expression in the HYP (171% of controls, ***$p<0.001$, Newman-Keuls test) and significant decreases in the CP and NA (62% of control, **$p<0.01$ and 70% of controls, *$p<0.05$, Newman-Keuls test, respectively). Thus, chronic inhibition of serotonin uptake can regulate PDYN expression in the HYP, CP, and NA. Since cocaine or GBR 12909 produced significant decreases in the HYP and cocaine also produced a significant increase in PDYN gene expression in the CP, regulation of PDYN gene expression by fluoxetine might be different from that by cocaine in this brain region. Then we examined the effects of selective norepinephrine uptake inhibitor nisoxetine on PDYN gene expression. This treatment produced significant increases in PDYN gene expression in the HYP, (183% of control, **$p<0.01$, Newman-Keuls test), NA (142% of control, **$p<0.01$, Newman-Keuls test) and hippocampus (124% of control, *$p<0.05$, Newman-Keuls test) and a significant decrease in the CP (69% of control, *$p<0.05$, Newman-Keuls test). These data suggest that nisoxetine affects PDYN gene expression and support a role for norepinephrine in the mechanism underlying the effects of chronic exposure to addictive or non-addictive drugs. Moreover, nisoxetine, as well as fluoxetine, decreases PDYN mRNA in the CP, in contrast to the up-regulation produced by cocaine. Thus, the inhibition of NE uptake alone cannot account for the cocaine-induced increase of PDYN gene expression. Cocaine, GBR 12909, fluoxetine and nisoxetine inhibited CREB phosphorilation in CP (57 ± 10 % $p<0.05$, 59 ± 11 % $p<0.05$, 62 ± 9 % $p<0.05$ and 51 ± 9 %, $p<0.05$), whereas no effect was observed in NA and HYP. On the contrary, cocaine and nisoxetine significantly induced CREB activation in the hippocampus (340 ± 49 %, $p<0.01$ and 300 ± 39 % $p<0.05$, respectively).These findings suggest that PDYN gene expression regulation by cocaine in the caudate putamen might be due to a combination of effects on two or three monoamine transporters, or to a mechanism unrelated to transporters inhibition. Furthermore modulation of prodynorphin gene expression may be due to CREB pathway involvement in CP and hippocampus.

EFFECTS OF 3,4-METHYLENEDIOXY-N-METHYLAMPHETAMINE (MDMA, 'ECSTASY') ADMINISTRATION ON THE DYNORPHINERGIC SYSTEM IN THE RAT BRAIN

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The prodynorphin system is implicated in the neurochemical mechanisms activated by psychostimulants. Exposure to different drugs of abuse can induce neuroadaptations in the brain and affect opioid gene expression. The present study aimed to examine the possibility of a common neurobiological substrate in drug addiction processes. We studied the effects of single or repeated (twice a day for seven days) 3,4-Methylenedioxy-N-methylamphetamine (MDMA, 'Ecstasy') administration on the gene expression of the opioid precursor prodynorphin, and on the levels of peptide dynorphin A in the rat brain. Acute (8 mg/kg, intraperitoneally) MDMA markedly raised prodynorphin mRNA levels in the prefrontal cortex (161.7 ± 13.3 % of control, ** $p \leq 0.01$), and in the caudate putamen (127.4 ± 3.2 % of control, ** $p \leq 0.01$), whereas it decreased gene expression in the ventral tegmental area (59.1 ± 4.7 % of control, ** $p \leq 0.01$). Chronic (8 mg/kg, intraperitoneally, twice a day for 7 days) MDMA increased prodynorphin mRNA in the nucleus accumbens (194.8 ± 20.3 of control, ** $p \leq 0.01$), hypothalamus (220.9 ± 30.9 % of control, ** $p \leq 0.01$) and caudate putamen (126.3 ± 7.9 % of control, * $p \leq 0.05$) and decreased it in the ventral tegmental area (58.9 ± 4.4 % of control, *** $p \leq 0.001$). Dynorphin A levels increased after chronic treatment in the ventral tegmental area (10.1 ± 0.5 pmol/g tissue; 166 ± 8 % of control, ** $p \leq 0.01$) and caudate-putamen(7.0 ± 0.9 pmol/g tissue; 175 ± 25 % of control, * $p \leq 0.05$) and decreased after acute treatment in the nucleus accumbens (5.2 ± 0.7 pmol/g tissue; 71 ± 14 % of control, * $p \leq 0.05$), prefrontal cortex (12.1 ± 2.1 pmol/g tissue; 48 ± 8 % of control, ** $p \leq 0.01$) and hypothalamus (31 ± 8 pmol/g tissue; 39 ± 10 % of control, ** $p \leq 0.01$)
These findings confirm the role of the dynorphinergic system in mediating the effects of drugs of abuse, such as MDMA, in various regions of the rat brain that may be important sites for the opioidergic mechanisms activated by addictive drugs.

ANALYSIS OF AMPHETAMINE, METAMPHETAMINE AND MDMA ("ECSTASY") IN HUMAN PLASMA AND URINE BY MEANS OF LIQUID CHROMATOGRAPHY WITH FLUORIMETRIC DETECTION

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Amphetamine (a-methylphenylethylamine) and its analogues such as methamphetamine (N,a-dimethylphenylethylamine) and MDMA (N,a-dimethyl-3,4-methylenedioxyphenylethylamine) are currently among the most well-known abuse drugs. They are used especially by young people as stimulants during the week-end (e.g. in discos), and are thus often referred to as "recreational drugs". Their mechanism of action is complex, but surely involves the release of catecholamines and serotonin from synaptic vesicles. Aim of this study is the development of a reliable analytical method for the simultaneous determination of amphetamine, methamphetamine and MDMA, and its application to the analysis of these compounds in human biological fluids, such as plasma and urine.Materials and Methods: Since the analytes show native fluorescence, liquid chromatography (HPLC) with fluorimetric detection was chosen for the purpose of separating and quantitating them. Separation was achieved on a C8 reversed-phase column using a phosphate buffer/acetonitrile (88:12) mixture (apparent pH = 2.8) as the mobile phase, flowing at 1 mL/min. Quinine was chosen as the Internal Standard (IS). Detection wavelengths were: lexc = 210 nm; lem = 300 nm for the analytes; lexc = 340 nm; lem = 420 nm for the IS. The sample pre-treatment procedure was carried out by means of solid-phase extraction (SPE) on hydrophilic-lipophilic balance cartridges. The cartridges were loaded with 250 μL of plasma or urine; the analytes were then eluted, dried and redissolved with 250 μL of mobile phase.Results: Under the described leading conditions, all analytes are baseline separated and detected within 13 minutes. Good linearity was obtained over different concentration ranges, according to the drug and the matrix. The sample pre-treatment procedure gave good extraction yields for all analytes, with mean recovery values ranging from 85 to 95% in plasma. Furthermore, the samples are devoid of interference from the biological matrices, thus good purification has been achieved. Conclusion: The method thus developed seems to be suitable for the determination of amphetamine, methamphetamine and MDMA in human plasma and urine.

EFFECT OF ETHANOL ON SYNAPTIC INPUT IN CEREBELLAR PURKINJE NEURONS.

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The cerebellum is classically associated with the control of body movement and coordination. Alcohol has been shown to alter coordination and motor skills and these effects contribute to a large number of fatal car accidents around the world. However little is known about the mechanism by which ethanol (EtOH) alters cerebellar functioning. We recently began to assess the effects of ethanol on synaptic transmission in the cerebellar cortex, where several neuronal types wire together in repetitive modules. We found that ethanol increases GABAergic transmission onto cerebellar granule cells by increasing the firing of the Golgi cells. We decided to extend our studies to Purkinje Cells (PC). The PC, the only output in the cerebellar cortex, receives two different types of excitatory synaptic inputs: the parallel fibers and the climbing fiber (CF). CF activation induces a massive release of glutamate in an all or none fashion. The stimulation of a CF elicits in the PC a large EPSP denoted as the complex spike (CS). The waveform of the CS is composed of an early phase and late phase. AMPA receptors and voltage gated sodium channels mediate the early phase, while the late phase is mediated, in part, by the activation of voltage gated calcium channels. We tested the effect of EtOH at CF-to-PC synapses in cerebellar slices that were prepared from adult rats. We performed patch-clamp electrophysiological experiments from PCs. CFs were activated with a stimulating electrode placed in the granule layer below the recorded PC. EtOH (50 mM) failed to affect the probability of glutamate release from the CF (we measured the excitatory postsynaptic current paired-pulse ratio). However, current-clamp experiments revealed that EtOH (50 mM) reversibly decreased the late phase area of the CS to 79 ± 10% of control (n=10), but was ineffective on the early phase. The mechanism by which EtOH affects the late phase of the CS is currently under investigation. We speculate that the EtOH-induced changes in the CS may have an impact in the PC physiology, and in turn alter information processing in the cerebellar cortex. Supported by NIH grant AA14973.

EFFECT OF FLUMAZENIL DURING ETHANOL WITHDRAWAL: A MOLECULAR AND FUNCTIONAL STUDY IN RAT CEREBELLAR GRANULE CELLS IN CULTURE

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Ethanol is a central nervous system depressant with a pharmacological spectrum of actions overlapping that of benzodiazepines known to act by enhancement ©-aminobutyric acid type A receptors (GABAAR). Different subtypes of GABAAR subunits confer distinct pharmacological properties and sensitivity to drugs. In this study we use cerebellar granule neurons and demonstrate that the gene expression and function of GABAAR receptors are altered during ethanol withdrawal. Cells were incubated for 5 days in the absence or presence of 100 mM ethanol and then the abundance of the GABAAR ⟨1, ⟨2, ⟨4 and ™ subunit mRNAs was determined with an RNase protection assay. Ethanol withdrawal increases the gene expression of the α2 and α4 GABAAR subunits and decreases the ⟨1, ™ and γ2 subunits. We, subsequently, investigated the effects of diazepam and flumazenil on ethanol withdrawal-induced molecular changes by incubating the cells with diazepam or flumazenil (10 μM) during withdrawal. The substitution of ethanol with diazepam or flumazenil antagonized the increase in gene expression of the ⟨4 subunit induced by ethanol withdrawal while failed to antagonize the upregulation of the ⟨2 subunit and the downregulation of the ⟨1, γ2 and ™ subunits. These ethanol withdrawal-induced molecular changes were accompanied by corresponding GABAAR functional changes. Patch-clamp registrations demonstrate that in ethanol withdrawn granule cells flumazenil potentiated the GABA-evoked Cl- currents consistently with the increased expression of the α4 subunit, while the modulatory action of zaleplon was reduced, consistent with the down-regulation of the α1 and ©2 subunits. The positive modulation of GABA-evoked Cl- currents by flumazenil was completely abolished when ethanol was substituted with diazepam or flumazenil during ethanol withdrawal. Our data demonstrate that diazepam and flumazenil are able to revert some molecular changes of the GABAAR induced by ethanol withdrawal suggesting that these molecular events play a pivotal role in the therapeutic action of these drugs in the treatment of the ethanol withdrawal condition.

ROLE OF FEEDING PEPTIDES IN ETHANOL INTAKE.

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It has been suggested that peptides involved in feeding/satiety control may also regulate ethanol intake. The relationship between ethanol and feeding/satiety peptides is also suggested by the finding that ethanol intake increases satiety self-rating in humans. The present study, therefore, evaluated the effect of CCK-8 and melanocortin receptor ligands on ethanol intake of male Marchigian-Sardinian alcohol-preferring (msP) rats. Both CCK-8 and MSH are well know to suppress feeding and induce satiety in rats, the first acting through a peripheral mechanism, while the latter acting at central sites of action. Intraperitoneal (ip) injection of CCK-8 (0, 0.5, 1, 2, 4, 8 μg/kg) produced a dose-related reduction of ethanol intake offered 2 hr/day to freely feeding and drinking msP rats, while intracerebroventricular injection of CCK-8 did not affect ethanol intake. Devazepide, a CCK-A receptor antagonist, blocked the effect of CCK-8 (4μg/kg) and, given alone, significantly increased voluntary ethanol drinking in msP rats. The dose of 4 μg/kg of CCK-8 also reduced blood alcohol levels when a fixed volume of ethanol (2 or 4 ml) was given by gavage. Chronic ip injection of CCK-8 (4 μg/kg) produced a reduction of ethanol intake without any significant sign of tolerance at doses that did not significantly modify water and food intake. Central injection of the melanocortin 3/4 receptor agonist, MTII, produced a decrease in ethanol consumption in msP rats but always associated to food intake reduction. The present data suggest that endogenous CCK selectively reduces ethanol ingestion, while MTII may reduce ethanol intake but only at doses that exert a general inhibitory effect of the ingestive behaviour.

PRESYNAPTIC NICOTINIC AND mGLU RECEPTORS INTERACTION IN THE MODULATION OF NORADRENALINE RELEASE FROM FROM RAT HIPPOCAMPAL SYNAPTOSOMES.

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It is well known that receptor-receptor interactions are present at the presynaptic receptor level. Data from our laboratory have previously shown functional interaction between NMDA and somatostatin receptors which coexist on the same noradrenergic nerve ending (1). In the present study we examined the interaction between nicotinic (nAch) and group I metabotropic glutamate (mGlu) receptors in the modulation of noradrenaline (NA) release from hippocampal nerve terminals prelabeled with [3H]-NA. Adult male rats were used and crude hippocampal synaptosomes were prepared as previously described (2). Nicotine (100⌈M) enhanced the basal release of [3H]-NA (basal release: 0.95±0.1 %; nicotine evoked release: 1.7±0.03 %) while the group I glutamate metabotropic agonist DHPG (100 ⌈M) did not produce any increase in NA release. However, it has been found that, when nicotine was added together with DHPG, the result being a potentiation of nicotine evoked [3H]-NA release (nicotine + DHPG evoked NA release : 2.0±0.05, $p<0.01$ versus nicotine). The EC50 of nicotine alone (1.80 ⌈M) did not differs when also DHPG was present (1.76 ⌈M). The effect of DHPG was counteracted by mGluR5 antagonist MPEP (1 ⌈M), but not by mGluR1 antagonist CPCOOET (5 ⌈M). The nicotinic evoked [3H]-NA release elicited in presence of DHPG was totally counteracted by mecamylamine (100 ⌈M).The effect of DHPG activation on the [3H]-NA release evoked by veratrine and ionomycin were also investigated. No synergistic response was observed when DHPG and ionomycin were both present in the superfusion medium, while DHPG and veratrine produced a synergistic effect.
In conclusion Nicotinic and GroupI Metabotropic Glutamate Receptors seem to coexist on NA terminals and synergistically potentiate [3H]-NA release.

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(1) Pittaluga et al., Neuropharmacology 41, 301-310, 2001
(2) Raiteri,L., Raiteri,M. (2000).Neurochem.Res.25:1265-1274

ROLE OF PRESYNAPTIC NICOTINIC AND PURINERGIC RECEPTORS IN THE MODULATION OF GLUTAMATE RELEASE FROM RAT CEREBROCORTICAL SYNAPTOSOMES.

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The co-storage and co-release of acetylcholine and nucleotides is well documented both in peripheral and central nervous system. However, little information are available on the interaction between cholinergic and purinergic signalling mechanisms at presynaptic level. The aim of the present study was to analyze the effect of nicotine and ATP in the modulation of the [3H]-D-Aspartate release from rat cortical synaptosomes superfused in conditions known to prevent indirect effects (1). Data from our laboratory have previously shown that nicotine (30μM) is unable to modify the glutamate basal release from rat cerebrocortical synaptosomes (2) while ATP (1mM) induce an exocytotic release of [3H]-D-ASP (0.76±0.04 % overflow). When ATP is added to nicotine there is a significative increase of [3H]-D-ASP overflow (1.07±0.06 %).
This synergistic effect is completly counteracted by Methyllycaconitine (10nM) (0.72±0.02%) and ⟨-Bungarotoxin (100nM) (0.75±0.02%).
It is conclude that presynaptic nAChRs and P2X receptor coexist on the cerebrocortical glutamatergic terminals and modulate in the synergic manner [3H]-D-ASP release.

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(1) Raiteri L., Raiteri M. (2000). Neurochem.Res.25:1265-1274
(2) Marchi M., Risso F. (2002). J.Neurochem.80(6):1071-1078

LINKAGE DISEQUILIBRIUM AND HAPLOTYPE ANALYSIS OF POLYMORPHISMS IN THE GABA RECEPTOR CLUSTER ON CHROMOSOME 4: ASSOCIATION STUDY.

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Recently data from association based genome scans provided key information about chromosomal regions harboring genes and allelic variants that may contribute to the genetic vulnerability to substance abuse. One of the regions (rSA3) on chromosome 4p12 was delineated by variants showing association with polysubstance abuse [1], linkage and family based association with alcoholism and brain oscillations [2, 3], and linkage with alcoholism [4]. Among other candidate genes rSA3 also contained a GABA receptor alpha cluster (GABRA2, GABRA4, GABRB1, and GABRG1), GABRA2 being the reported center of association [2]. In this study, we have examined single nucleotide polymorphisms in this region, constructed linkage disequilibrium (LD) map and haplotypes spanning GABRA2 gene and examined association of this haplotypes with polysubstance abuse and alcoholism in three independent populations. The results presented here indicate that there are SNPs and haplotypes in and around GABRA2 and GABRG1 with alleles unevenly distributed between control groups and substance abuser groups in two populations of polysubstance abusers and matched controls: Caucasian and African American. Our results provide no evidence that the variations in the GABRA2 gene affect both alcoholism and drug abuse. We found a five-SNPS and a two-SNPs haplotype, both including rs279871, marginally associated with alcoholism and drug abuse respectively. Our findings also show a very strong LD with alcohol dependence in the region between rs573400 and rs279867.

1) Uhl GR, et al. (2001) Am. J. Hum. Genet. 69: 1290-1300.
2) Edenberg HJ, et al. (2004) Am. J. Hum. Genet. 74: 705-714.
3) Porjesz B, et al. (2002) Proc. Natl. Acad. Sci. USA. 99: 3729-3733.
4) Long JC, et al. (1998) Am. J. Med. Genet. 81: 216-221.

ALLELIC ASSOCIATION ANALYSIS OF THE μ -OPIOID AND CANNABINOID RECEPTOR GENES WITH HEROIN ADDICTION

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Twin, family and adoption studies have indicated that vulnerability to substance abuse and addiction may be a partially inherited condition with strong influences from environmental factors as well. In particular, heroin was shown to have a larger genetic influence when compared to other substances of abuse. Two of the most reasonable candidates for opioid dependence were the μ opioid (OPRM1) and cannabinoid (CNR1) receptor genes. Several opioids' actions such as euphoria, analgesia, and withdrawal are mediated through the μ opioid receptors and play a key role in the positive and negative reinforcing properties of heroin and other opioids. The endogenous cannabinoid system is thought to be an important neuromodulator in motivation, reward and motor control systems. Observations using animal models suggest that modulation of endogenous central cannabinoid signaling in mesolimbic pathways may be a component of the addiction process, particularly via interaction with opioid and dopaminergic systems. The present study examined the involvement of a polymorphism A118G in exon 1 of the OPRM1 and G1359A in codon 453 of the CNR1 in 100 Sardinian heroin-dependent and 129 control subjects. All subjects with heroin dependence were recruited from the Addiction Therapy Unit (SER.T, ASL n.8, Cagliari). After obtaining written informed consents, individuals were diagnosed as having opiate dependence using DSM-IV criteria following a semi-structured clinical interview. SNPs at OPRM1 and CNR1 genes were analyzed by PCR/RFLP. Statistical analysis of the genotype and allele frequency differences between opioid-dependent and control subjects for each of the polymorphisms studied yielded P values in the range of 0.18–1. A positive association was found between allele A of the CNR1 and a subgroup of heroin addicted without any other drug abuse diagnosis ($p = 0.02$, OR= 2.9). This polymorphism is a silent mutation which can be suspected of being in strong Linkage Disequilibrium with another functional mutation in the gene, as otherwise the quantity of gene expression would be affected. In conclusion, in spite of the small size of the sample examined and the need of replication in other types of population, our study suggests that the CNR1 may play a role in a subgroup of heroin addict.

MK-801 NEUROTOXICITY: BEHAVIOURAL AND HISTOLOGICAL EFFECTS IN RODENTS.

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Nowadays the well known *aril-alchil-amine*, as ketamine and PCP (phencyclidine) and other NMDA antagonists, are repeatedly in use among young people as inhalant drugs but the effects of this employment is not yet been explored. These compounds as well as other NMDA antagonists (i.e. MK801) have shown to cause pathomorphological changes in the posterior cingulated and retrosplenial cortex in rat brains. The main effect induced by a moderate acute administration is the reversible development of intracytoplasmic vacuoles in the layers III and IV of the retrosplenial cortex whereas an high dose cause neuronal death in the same area. The intensity of these effects could change depending on dose, age and sex (females appear to be more sensitive) and share several features with some neurodegenerative disorders such as the Alzheimer's disease and schizophrenia.
The neurotoxic effects of NMDA antagonists have only been reported once in a species other than rats (mice) and species differences cannot be ruled out. Therefore this study was undertaken to evaluate the neurotoxicity of NMDA antagonists in another rodent model such as hamster comparing it with the behavioural and histological picture present in rats. First we performed behavioural studies employing female Sprague Dawley rats and female Sirian hamster testing a high dose (10 mg/kg) of MK-801. We observed a neuroplegic picture more intense in hamsters than in rats and hamsters also presented convulsive phenomena completely absent in rats. These behaviors persisted for 3-6 hours after the treatment and were coupled to a significant reduction in body weight. On the basis of these behavioural results we verified the presence of neurodegeneration after MK-801 treatment using Fluoro-Jade B, a high affinity fluorescent marker for the neuronal degeneration that allows to detect specifically the entire degenerating neurons including cell body, dendrities, axon. The coloration showed a widespread degeneration within the retrosplenial cortex and hippocampus of the rats examinated also observed in hamster under the same experimental condition. As espected we found a large number of death cells in the retrosplenial cortex of rats after MK-801 acute treatment demonstrating that aril-alchil-amine compounds could produce an irreversible neurotoxic effect. In the hamster the neurotoxicity appears with a different region specificity. These data suggest that these substances can be dangerous also following sporadic administrations and the emerging picture resemble that present in some neurodegenerative disorders.

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IN VITRO PHARMACOLOGICAL PROFILE OF THE NOVEL DELTA/MU OPIOID RECEPTOR LIGAND H-Dmt-Tic-Gly-NH-CH2-Ph (UFP-505)

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Several lines of evidence obtained with receptor antagonists, oligo antisense, peptide and receptor knockout mice indicate that delta opioid peptide (DOP) receptor activation is involved in development/expression of tolerance to the analgesic effects of opioids. Thus, an opioid receptor ligand able to activate the mu opioid peptide (MOP) receptor and simultaneously block the DOP receptor may induce analgesic effects with reduced tolerance.
In the present study, we investigated the in vitro pharmacological profile of a novel opioid receptor ligand H-Dmt-Tic-Gly-NH-CH2-Ph (UFP-505). In receptor binding experiments, performed on membranes of CHO cells expressing the human recombinant opioid receptors, UFP-505 displayed a 30 fold higher affinity for DOP (pKi 9.90) than for MOP (pKi 8.30) sites. In CHOhMOP GTPγS stimulation experiments, UFP-505 behaved as a full agonist (pEC50 7.70), while in CHOhDOP membranes the compound antagonized Leu-enkephalin stimulatory effect showing a pKB value of 10.39. In the electrically stimulated mouse vas deferens (a DOP receptor preparation) UFP-505 up to 100 nM did not evoke any effect per se but shifted to the right the concentration response curve to the DOP selective agonist deltorphin I; a pA2 value of 9.13 was obtained from these experiments. On the other hand, in the electrically stimulated guinea pig ileum (a MOP receptor preparation) UFP-505 mimicked the inhibitory effects of the selective MOP receptor agonist dermorphin (pEC50 9.41), being however 100 fold less potent (pEC50 7.49). The inhibitory effects of UFP-505 were sensitive to non selective (naloxone; pA2 8.8) and selective (CTOP; pA2 8.29) MOP receptor antagonists. Similar results were obtained with the two antagonists against dermorphin. Collectively, the present data suggest that UFP-505 behaves as a DOP antagonist/MOP agonist; this peptide can be used in future studies as a chemical template for the design and synthesis of novel opioid receptor ligands with analgesic activity and reduced tolerance liability.

[Nphe1,Arg14,Lys15]NOCICEPTIN-NH2 BLOCKED THE EXPRESSION OF OPIOID TOLERANCE.

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Background. The appearance of tolerance to chronic use of drugs is a characteristic of all the opioid analgesics and one the major problems in their clinical use (1). It is now well established that nociceptin/NOP receptor (Nc/NOPr) system is involved in the induction of dependence and development of tolerance to opiate actions. Aim of the study was to investigate whether intracerebroventricular (i.c.v.) administration of the antagonist for NOPr [Nphe1,Arg14,Lys15]nociceptin-NH2 UFP-101 (2) would affect the expression of tolerance to the antinociceptive effect of morphine. Methods. Saline (Ve 3µl i.c.v.) or UFP-101 (2 nmol/3µl i.c.v.) was injected (by cannulae surgically implanted) on the day 5, 10 min after the last administration of morphine (M 7 mg/kg/5 days intraperitoneally i.p.) or saline (Ve 5 ml/kg/5 days i.p.). The tail flick test (TF) was used as a nociceptive assay. Results. UFP-101 administered on the day 5, did not modify the nociceptive responses in animals treated chronically with saline but inhibited the expression of tolerance to the antinociceptive effect of morphine in mice rendered tolerant to the opioid [mean ± s.e. of latency of response in sec: Ve Day 1 6.7 ± 0.4; Day 5 7.0 ± 0.5; M Day 1 12.7 ± 0.6; Day 5 8.6 ± 0.3*; UFP+M Day 1 12.2 ± 0.6; Day 5 10.9 ± 0.8°; ANOVA *P<.01 vs Day 1 and ° P<.01 vs M] Conclusion. It has been hypothesized that the increase of NOPr expression and nociceptin levels observed in morphine tolerant animals reduced the analgesic effects of morphine, contributing to opioid tolerance. Our results might suggest that UFP-101 inhibited the expression of opioid tolerance by blocking the anti-analgesic effect of endogenous nociceptin.

1) Martin W.R. (1983) Pharmacol. Rev. 36 :283-323.
2) Calo' G. & al., (2002) Br. J. Pharmacol. 136:303-311.

NOCICEPTIN REDUCED THE INHIBITION INDUCED BY [Nphe1,Arg14,Lys15]NOCICEPTIN-NH2 ON THE EXPRESSION OF OPIOID TOLERANCE.

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Background. The development of tolerance and physical dependence are known effects of opioid analgesics (1). Despite extensive research in this area the exact mechanisms by which these processes occur remain unknown. Since the activation of the anti-opioid nociceptin/NOP receptor (Nc/NOPr) system might be involved in the induction of opioid dependence and tolerance, aim of this study was to investigate whether intracerebroventricular (i.c.v.) administration of the NOPr agonist nociceptin (Nc) or the antagonist [Nphe1,Arg14,Lys15]nociceptin-NH2 UFP-101 (2) affected the expression of tolerance to the antinociceptive effect of morphine. Methods. Saline (Ve 3 µl i.c.v.), Nc (1 nmol/3µl i.c.v.) and UFP-101 (UFP 2 nmol/3µl i.c.v.) were injected (by cannulae surgically implanted) alone or in combination, on the day 5, 10 min after the last administration of morphine (M 7 mg/kg/5 days intraperitoneally i.p.) or saline (Ve 5 ml/kg/5 days i.p.). The tail flick test (TF) was used as a nociceptive assay. Results. Nc and UFP-101 administered on the day 5, did not modify the nociceptive responses in animals treated chronically with saline. Nc further reduced opioid antinociception in morphine tolerant animals. On the contrary UFP-101 inhibited the expression of opioid tolerance in mice rendered tolerant to morphine. When Nc was injected together with UFP-101 reduced the inhibition of UFP-101 on the expression of morphine tolerance [mean ± s.e. of latency of response in sec: M Day 1 12.7 ± 0.6; Day 5 8.6 ± 0.3**; UFP+M Day 1 12.2 ± 0.6; Day 5 10.9 ± 0.8; Nc+UFP+M Day 1 11.8 ± 0.8, Day 5 8.7 ± ; 0.6** °; ANOVA **P<.01 vs Day 1 and ° P<.05 vs UFP+M] Conclusion. Our results suggest that UFP-101 inhibited opioid tolerance by blocking NOPr.

1) Jage J., (2005) Eur. J. of Pain 9: 157-16.
2) Calo'G. & al., (2002) Br. J. Pharmacol. 136: 303-311.

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