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IN NEUROFARMACOLOGIA

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IL PROMETEO

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Direttore:
Prof. Filippo Drago

DOTTORATO
INTERNAZIONALE
IN
NEUROFARMACOLOGIA

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IL CEINGE: A NAPOLI UN CENTRO DI ECCELLENZA PER LE TECNOLOGIE AVANZATE

Carmen Mazzola e Michel Hamon (Parigi) alla consegna dei Travel Awards dell'European College of Neuropsychopharmacology

Sono trascorsi quasi otto anni da quel Novembre dell'anno 2002, quando raggiungevo il tanto agognato traguardo: la laurea in Medicina. Già alla fine del V anno del corso di laurea, iniziavo a frequentare il laboratorio di farmacologia comportamentale del prof. Filippo Drago, mio mentore allora come ora. Ogni giorno che trascorrevo in laboratorio, per svolgere gli esperimenti per la mia tesi di laurea, riguardante il coinvolgimento dei recettori per i cannabinoidi nei processi cognitivi, cresceva la passione per quello che imparavo, finché ho deciso che la ricerca sarebbe stata la mia scelta professionale dopo la laurea. Infatti, nel 2007 ho conseguito la Specializzazione in Farmacologia, sempre presso l'Università di Catania,considerato la possibilità di accedere al Dottorato Internazionale in Neurofarmacologia della stessa Università.

In quel periodo, tuttavia, ho sospeso il mio percorso formativo a Catania ed ho vissuto un'importante esperienza scientifica della durata di 2 anni negli USA, presso i laboratori del National Institite of Drug Abuse, NIDA (affiliato al National Institute of Health, NIH) a Baltimora, dove ho svolto una serie di esperimenti di learning and memory che

hanno prodotto diverse pubblicazioni e l'avvio di un progetto di ricerca presso la Jhons Hopkins, e l'implicazione sia dei cannabinoidi che dei recettori PPAr nella patogenesi della Malattia di Alzheimer. Conseguita la Specializzazione in Farmacologia, il percorso era tutto ancora in crescita, ma ho superato il concorso di ammissione al Dottorato Internazionale in Neurofarmacologia, sempre sotto la direzione del prof. Drago.

Durante il primo anno, ho portato a compimento gli esperimenti condotti negli USA, presentandone alcuni dati al Congresso dell'European College of Neuropsychopharmacology (ECNP) a Barcellona nel 2008. Allo stesso Congresso sono risultata vincitrice per la seconda volta un travel award che mi è stato consegnato dal prof. Michel Hamon, psicofarmacologo di Parigi (fotografia). In quel momento, si è riproposta la possibilità di uno stage presso un laboratorio estero od italiano. Ho iniziato a documentarmi, sempre più convinta di volermi mettere in gioco ancora una volta, e possibilmente in una realtà italiana. Perché i cervelli migliori dovevano essere sempre quelli 'in fuga'? Certamente volevo continuare a lavorare nel mio filone di ricerca, che ormai da oltre 5 anni era quello delle Neuroscienze comportamentali e della Farmacologia. Mi avevano parlato di un centro di ricerche a Napoli, il CEINGE.

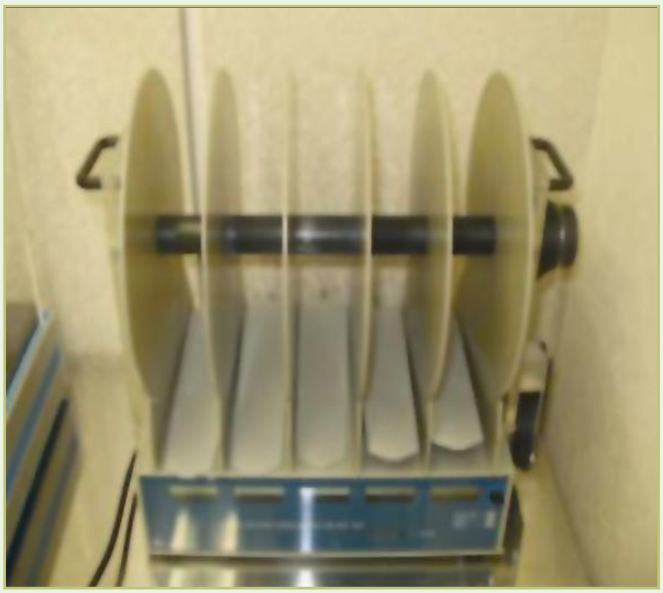
Ho cercato informazioni sul web, ed ho appreso che in questo centro si svolgevano ricerche nell'area della neurobiologia del comportamento. Ho deciso che era lì che volevo andare. Mi sono messa in contatto con il prof. Alessandro Usiello, direttore del laboratorio di neuroscienze del CEINGE, che dopo aver esaminato il mio curriculum ha accettato di ospitarmi ed integrarmi, fino a tutt'oggi, nel suo laboratorio di ricerca.

Anno	Articoli	Impact Factor
fino al 2003	161	714.888
2004	35	179.792
2005	54	326.387
2006	76	348.784
2007	75	342.169
2008	78	349.441
Totali	**479**	**2,261.461**

In questi ultimi due anni, il progressivo aumento del numero dei Gruppi di Ricerca del CEINGE e quindi delle linee di ricerca attive presso il centro, hanno dato forte impulso alla produzione scientifica sia in termini quantitativi, che qualitativi. In relazione all'ultimo triennio sono stati pubblicati oltre 200 articoli scientifici su riviste specializzate internazionali. (per i dettagli cliccare sull'anno).

Il CEINGE (dall'acronimo di "Centro di Ingegneria genetica", poichè alla sua istutizione quest'attività di ricerca era stata identificata come quella corrispondente il Centro stesso) è una struttura moderna, qualificata per la ricerca nel campo della biologia molecolare e delle biotecnologie avanzate; consta di servizi di alta tecnologia a supporto della ricerca nei settori di competenza, basati su piattaforme tecnologiche di ultima generazione di genomica e post-genomica.

Esso rappresenta un centro di alta formazione nelle biotecnologie avanzate e nella medicina molecolare; si propone la promozione della diffusione della cultura scientifica e tecnologica per favorire gli scambi di conoscenze tra gli Enti legati al settore della ricerca e per lo sviluppo delle biotecnologie e la produzione di beni (prototipi, reagenti, fine chemicals, molecole farmacologicamente e biologicamente attive, su piccola scala) nel campo delle biotecnologie avanzate. In merito alle mie competenze, ho approcciato nuove tecniche comportamentali, come novel object recognition, il fear conditioning, il social defeat, ed ho anche applicato importanti nozioni di biologia molecolare.



Apparato per il rotarod, necessario per testare le capacità di coordinazione motoria nel topo.

Nel dettaglio, ho partecipato a studi riguardanti il ruolo del D-aspartato nei processi cognitivi e nell'invecchiamento, guadagnando un co-authorship nel paper 'Increased d-aspartate brain content rescues hippocampal age-related synaptic plasticity deterioration of mice', pubblicato su Neurobiology of Aging, ed alla identificazione fenotipica di un modello transgenico murino per l'ADHD. In questo ultimo studio, abbiamo riscontrato come una mutazione puntiforme relativamente al sito di legame per la cocaina sul trasportatore della dopamina, DAT, sia sufficiente per il verificarsi di importanti e reversibili condizioni per rapportare questo fenotipo a quello dell'iperattività.

Ho presentato i dati principali di questa ricerca al Congresso della Society for Neuroscience del 2009 a Chicago, e saranno anche pubblicati in un articolo attualmente in corso di realizzazione.

La mia esperienza sia scientifica che umana si va sviluppando con grande soddisfazione da parte mia, poiché oltre ad accrescere il mio profilo di ricercatore, essa mi ha messo in contatto con una realtà sociale molto variegata. Il CEINGE, infatti, ospita un gran numero di giovani e validi ricercatori, italiani ed stranieri.

Oltre all'attività di laboratorio, mi sto molto dedicando alle implicazioni cliniche della Neurofarmacologia. Da diversi mesi, infatti, svolgo pratica clinica in una clinica neuropsichiatrica napoletana, comprovando che la ricerca di base non deve essere puro nozionismo, ma anzi fornisce gli elementi fondamentali per un corretto management farmaco-terapeutico dei pazienti.

A compimento del mio ciclo di Dottorato, ormai prossimo alla conclusione, al CEINGE ho sicuramente arricchito il mio bagaglio scientifico e culturale, ma ho anche acquisito nuove competenze ampliando le papabili prospettive lavorative.

Carmen Mazzola

PhD abstract

Posttranscriptional Regulation of VEGF in Diabetic Retina: Role of the RNA-binding protein

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PURPOSE. To investigate whether VEGF expression is regulated at posttranscriptional level in diabetic retinopathy through the mRNA-stabilizing protein HuR and via PKCβ activation. METHODS. Diabetes was induced in rats by streptozotocin (STZ) injection. Animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Retinal tissues were processed to detect PKCβI, PKCβII, VEGF and HuR contents, as well as HuR phosphorylation. Immunoprecipitation coupled to western blotting or RT-PCR was employed to evaluate HuR phosphorylation and its binding to VEGF mRNA, respectively. Statistical analysis was performed by ANOVA followed by an appropriate post hoc comparison test. RESULTS. Following experimental diabetes PKCβI and PKCβII levels were statistically ($p<0.01$) increased compared to sham; we also showed a PKC-mediated phosphorylation/activation of HuR. These effects were statistically ($p<0.01$) blunted by the co-administration of a PKCβ inhibitor (INH). A specific binding between HuR protein and VEGF mRNA was also evaluated in RiboNucleoProteic complexes from sham and diabetic animals indicating no significant difference in all retinal tissues. The PKCβ/HuR activation was accompanied by statistical ($p<0.01$) enhanced VEGF protein expression that was blunted by the PKCβ inhibitor. However, the total VEGF mRNA levels did not change following diabetes (mean ± S.E.M. of Ct values: sham = 28.8 ± 0.86; STZ = 28.5 ± 0.84; STZ+ INH = 28.7 ± 1.02; n=6). CONCLUSIONS. The present findings indicate that, following diabetes, there is an higher phosphorylation of HuR, a nucleo-cytoplasmic shuttling protein, by PKCβ resulting in the activation of HuR itself that then targets VEGF mRNA, finally leading to an increased amount of the correspondent VEGF protein. These data, along with the observation that VEGF mRNA shows no changes following diabetes, suggest that VEGF may be regulated mainly at posttranscriptional level.

Acknowledgments: This work was supported by a grant from MIUR - PRIN 2007.

Note

Lo studio è stato presentato al Congresso Internazionale ARVO (Association for Research and Visual Science) che si è tenuto a Fort Lauderdale, FL, USA dall'1 al 6 Maggio 2010.

The β_3 adrenoceptor agonist, amibegron counteracts behavioral and neurobiological changes induced by acute and repeated restraint stress

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It has been demonstrated that the activation of β3 adrenoceptors following administration of the agonist amibegron (SR58611A), exerted both anxiolytic and antidepressant effects in behavioral animal models. The present experiment was made to assess the antidepressant effect of amibegron in male Wistar rats tested in the forced swim test (FST), an experimental model widely used for preclinical studies on novel antidepressant drugs. Furthermore, the hippocampal expression of CREB, BDNF and Bcl-2/Bax ratio proteins was evaluated by Western Blot analysis. Wistar rats received acutely or repeatedly (once a day for 7 days), intraperitoneally (i.p.) injection of amibegron (1, 5 and 10 mg/kg), tricyclic antidepressant (TCA) clomipramine (50 mg/kg), selective serotonin reuptake inhibitor (SSRI) citalopram (15 mg/kg) or vehicles, respectively. The influence of stress-related conditions was studied in rats subjected to acute (4 h) or repeated (4 h/day for 7 days) restraint stress, made prior to the FST procedure. Both acute and repeated administration of amibegron (5 and 10 mg/kg) decreased the immobility time both under basal conditions and after stress exposure. In non stressed animals, amibegron (10 mg/kg) increased the expression of BDNF and CREB proteins ($p<0.05$) as well as of BDNF and Bcl-2/Bax ratio ($p<0.05$; $p<0.01$) after acute or repeated treatment, respectively. Both acute and repeated restraint stress procedure increased the immobility time in FST, in both vehicle and amibegron (1mg/kg/day) injected stressed animals in comparison to control NST group ($p<0.05$). Furthermore, rats showed lower BDNF and Bcl-2/Bax ratio proteins expression in control stressed animals compared to control NST group ($p<0.05$; $p<0.01$). Opposite effect was found in CREB expression, since it was lower or higher after acute or repeated stress procedure, respectively. Amibegron injection was found to counteract stress-induced behavioral and neurobiological changes. These results suggest that the pharmacological stimulation of β3 adrenoceptor may be a therapeutic target for the treatment of stress-related disorders.

Tools for measuring clinical effectiveness in schizophrenia

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EUROPEAN FRONTIERS IN NEUROPSYCHOPHARMACOLOGY

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Only recently success criteria became more ambitious and include a more thorough consideration of negative symptoms and cognitive dysfunction. The most important change within the last two decades, associated with the development of atypical antipsychotics subsequent to the development of clozapine, is the long overdue consideration of the patient's perspective. His/her quality of life or subjective well-being was neglected for a long time. One reason was the prejudice that schizophrenic patients are not able to self-rate well-being or quality of life.

Another reason was the belief that such data are not necessary because the psychiatrists' perspective, "objective" psychopathology, includes these domains. However, recent data indicate that in addition to the positive influence of a good relationship between doctor and patient, the subjective experience of antipsychotic treatment is a major predictor of compliance. In addition to the distressing motor symptoms that often accompany treatment with typical or first-generation antipsychotics, marked adverse effects on drive and emotion may also be experienced. Patients report a reduced quality of life with restricted emotionality, straight thinking and spontaneity, a syndrome very similar to negative symptoms of schizophrenia.

Among other scales, a self-report instrument has been constructed to evaluate "subjective well-being under neuroleptics" (SWN). This scale was used in numerous open and controlled trials, indicating: a) schizophrenic patients, if no longer acutely psychotic or suffering from severe cognitive deficits, are able to reliably assess their subjective well-being, b) high SWN is correlated with high compliance, c) atypical antipsychotics increase SWN, d) individual improvements of SWN and of PANSS are not strongly related (r=-.30 - -.40), and e) dopamine D2 receptor blockade is highly correlated to reduced SWN (r = .66 - .76).

Recent trials reveal the relevance of early improvement of subjective well-being: In a 12-week trial 95% of those with early subjective response (within 4 weeks) showed later subjective and/or psychopathological improvement, but only 9% without early subjective response showed later improvement. In another 3-year trial again psychopathological response as well as symptomatic and functional remission were not only related to young age and treatment with atypical antipsychotics, but mostly to early (within the first 3 months) subjective improvement. Moreover in a five year trial of first episode patients, marked improvement of SWN within the first 6 weeks of antipsychotic treatment was found to be related to enduring remission, while early improvement of PANSS did not predict outcome. Another new success criterion is insight into illness, its improvement is associated with improved compliance. However, recent cross-sectional studies report that good insight is related to poor quality of life. Longitudinal studies are needed to investigate the complex interactions, maybe mediated by stigma. Patients with high subjective stigma have also a very high negative correlation between insight and reduced quality of life.

In addition to simple outcome criteria such as all cause discontinuation of antipsychotic treatment, new treatment goals focus on social and functional outcomes, defined as remission or recovery. Data on the temporal patterns of improvement of psychopathology, quality of life and social functioning indicate that these different domains show similar early improvement if the antipsychotic drug is successful.

Pituitary adenylate cyclase-activating peptide, vasoactive intestinal peptide and receptors expression in the retina of streptozotocin-injected rats

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SOCIETY FOR NEUROSCIENCE

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PURPOSE: Both pituitary adenylate cyclase-activating peptide (PACAP) and the closely related vasoactive intestinal peptide (VIP) have been shown to exert protective actions in developing retinas. However, a role for both peptides in early diabetic retinopathy remains to be elucidated. In the present study we investigated whether PACAP/VIP peptides and receptors expression in the retina might be affected during the early stages of streptozotocin (STZ)-induced diabetic retinopathy. Furthermore, we analyzed the effect of an intravitreal injection of PACAP peptide on apoptosis.
METHODS: Diabetes was induced in Wistar rats by a single STZ intraperitoneal injection. PACAP/VIP peptides and receptors expression in healthy and early diabetic retinas were assayed both after 1 and 3 weeks by quantitative real-time PCR, Western blot and immunohistochemical analyses. A group of diabetic rats were subjected to either a single intravitreal injection of PACAP peptide (1pM/µL) or saline solution in the contralateral eye after 1 week. Changes in Bcl2 and cleaved-caspase 3 expression levels were then evaluated in both PACAP- and saline-injected eyes after 3 weeks.
RESULTS: VPAC1 and VPAC2 receptors, as well as PACAP and VIP peptides mRNA levels were transiently induced by STZ-treatment within 1 week. These findings were confirmed at the protein level both by immunoblot and immunohistochemistry, hence showing that no changes in receptors and peptides distribution occurred. After 3 weeks, PAC1, VPAC receptors and related peptides expression levels were remarkably reduced in diabetic as compared to healthy rat retinas. Concurrently, STZ-treatment significantly downregulated the expression of the antiapoptotic gene Bcl2 and upregulated the expression of the proapoptotic effector cleaved-caspase 3, suggesting that STZ-induced diabetes promotes the activation of the intrinsic apoptotic pathway in retinas. Interestingly, this effect was partially counteracted by the single PACAP intravitreal injection within 2 weeks, thereby causing a significant induction of Bcl2 gene and significant reduction of cleaved-caspase 3 expression levels in PACAP- as compared to saline-injected diabetic retinas.
CONCLUSIONS: The initial transient upregulation of PACAP/VIP receptors and peptides and their subsequent downregulation in STZ-treated rat retinas suggests the activation of an endogenous PACAP- or VIP-mediated protective mechanism, probably through an autocrine/paracrine loop, which is not sufficient to counteract STZ-induced detrimental effects on retinas. The data also supports PACAP peptide as an effective antiapoptotic agent in the therapy for diabetic retinopathy.

Acknowledgments: This work was supported by a grant from MIUR - PRIN 2007

Note

Lo studio dell'abstract sarà presentato al meeting annuale della SfN (Society for Neuroscience) che si terrà a San Diego, CA, USA dal 13 al 17 novembre 2010

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Saturday July 10th, 2010

10.30-11.15	Registration
11.15-11.30	Opening remarks **Filippo Drago (Italy)**
11.30-12.30	**Opening lecture** (introduced by Filippo Drago) Depression in schizophrenia: new perspectives for the treatment **Stuart Montgomery (United Kingdom)**
12.30-13.00	Welcome reception

Parco degli Aragonesi Hotel - Viale Kennedy, 2 (Catania)

Psychotic disorders: epidemiology and genetics
Introduced and moderated by Alessandro Rossi (Italy)

16.00-17.00	Psychotic disorders: epidemiology and risk factors **Mauro Carta (Italy)**
17.00-18.00	The genetics of bipolar spectrum disorders **Alessandro Serretti (Italy)**
18.00-18.30	Discussion
18.30-19.30	Genetics of schizophrenia: new insights from new approaches **Massimo Gennarelli (Italy)**
19.30-19.40	Questionnaire