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THE FINNISH-ESTONIAN MEETING OF NEUROPHARMACOLOGY

Viikki Biocenter, Helsinki, Finland, August 18-19, 2000

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THE FINNISH-ESTONIAN MEETING OF NEUROPHARMACOLOGY

August 18-19, 2000, Viikki Biocenter 2, Helsinki, Finland

Program and abstracts

Contents

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WELLCOME TO THE FINNISH-ESTONIAN MEETING OF NEUROPHARMACOLOGY

The organizers have a great pleasure to wellcome you to the first joint meeting of Finnish and Estonian Pharmacological Societies. The focus of the meeting is on neuropharmacology, and it consists of invited lectures, oral communications and poster sessions. Social programme includes a dinner sponsored by Algol OY. The meeting of the Finnish Pharmacological Society will be held on Friday, August 18, at 18.00. **The deadline for registration is extended to the August 8, 2000**. Registration is required for attending the dinner. For the registration, please visit the web site www.helsinki.fi/jarj/farmakologia, or contact Petteri Piepponen (e-mail petteri.piepponen@helsinki.fi) or Tiina Seppä (phone 09-191 59459, e-mail tiina.seppa@helsinki.fi).

The organizers:

Division of Pharmacology and Toxicology,
Department of Pharmacy, University of Helsinki
Finnish Pharmacological Society
Estonian Pharmacological Society
Finnish Graduate School of Pharmaceutical Sciences

SCIENTIFIC PROGRAM

Friday, August 18

9.00 ***Opening remarks***
Liisa Ahtee, University of Helsinki

9.15 ***Catechol-O-methyl transferase (COMT): gene, two proteins and new COMT inhibitors***
Pekka Männistö, University of Kuopio

10.00 ***Distribution of catechol- O-methyl transferase (COMT)***
Ilkka Reenilä, University of Helsinki

10.20 ***Inhibition of COMT by second-generation COMT inhibitors: in vitro and ex vivo studies***
Markus Forsberg, University of Kuopio

10.40 Coffee and mounting of posters

11.10 ***Effect of NMDA/glycine site antagonist L-701,324 on cocaine-stimulated dopamine release in the rat nucleus accumbens***
Toomas Kivastik, University of Tartu

11.30 ***Effects of μ-opioid receptor agonists on nigrostriatal dopamine system of alcohol-preferring AA and alcohol-avoiding ANA rats***
Aapo Honkanen, University of Helsinki/University of Turku

11.50 ***Effects of repeated administration of cocaine and morphine on nigrostriatal and mesolimbic dopamine in AA and ANA rats***
Janne Mikkola, University of Helsinki

12.10 Lunch and posters

14.15 ***Antidepressants and serotonin receptors***
Lembit Allikmets, University of Tartu

15.00 ***Apomorphine-induced aggressive behaviour in adult male Wistar rats: Implication to monoaminergic neurotransmission***
Vallo Matto, University of Tartu

15.20 Coffee and posters

16.00 ***Subtype selective alpha-2-adrenoceptor agents; can we predict their effects?***
Ewen McDonald, University of Kuopio

16.30 ***Structural determinants of ligand binding to alpha-2-adrenoceptors***
Anne Marjamäki, University of Turku

16.50 ***Deramciclane - preclinical evidence of anxiolytic activity***
Aavo Lang, University of Tartu

17.30 ***Sponsor's address of welcome***
Olavi Oinonen, Algol Oy

18.00 Meeting of the Finnish Pharmacological Society

19.00 Dinner at the restaurant Viikinkartano

Saturday, August 19

9.00 ***The influence of nicotine on mesoaccumbens dopamine - a window on the neurobiology of dependence?***
David Balfour, University of Dundee

9.45 ***Comparison of the effects of epibatidine and nicotine on the output of dopamine in the dorsal and ventral striatum of freely-moving rats***
Tiina Seppä, University of Helsinki

10.05 ***Brain monoamines and locomotor activity in mice during chronic oral nicotine administration and its withdrawal***
Helena Gäddnäs, University of Helsinki

10.25 ***Nicotinic effects of cotinine***
Petri Vainio, University of Helsinki

10.45 Coffee

11.15 ***Infection and cardiovascular disease***
Ilari Paakkari, University of Helsinki

11.45 ***Demonstration of endothelin ET_B-receptors in the nerves innervating bovine retractor penis muscle***
Ulla-Mari Parkkisenniemi, University of Helsinki

12.05 Lunch and posters

13.30 ***New strategies in the treatment of neurodegenerative disorders***
Alexander Zharkovsky, University of Tartu

14.00 ***Nitric oxide: good, bad and ugly in brain?***
Pekka Rauhala, University of Helsinki

14.30 ***The effects of the nitric oxide synthase inhibitors on the behaviour of small platform stressed mice in the plus-maze test.***
Paavo Pokk, University of Tartu

14.50 ***Concluding remarks***
Raimo Tuominen, University of Helsinki

Abstracts of the oral presentations

CATECHOL-O-METHYLTRANSFERASE (COMT): GENE, TWO PROTEINS AND NEW COMT INHIBITORS

Pekka T Männistö
University of Kuopio, Department of Pharmacology and Toxicology

COMT O-methylates catecholamines and other compounds having a catechol structure. The general function of COMT is the elimination of biologically active or toxic catechols and some other hydroxylated metabolites. COMT also acts as an enzymatic detoxicating barrier between the blood and other tissues shielding against the detrimental effects of xenobiotics. COMT may serve some unique or indirect functions in the kidney and intestine tract by modulating the dopaminergic tone. The same may be true in the brain: COMT activity may regulate the amounts of active dopamine and noradrenaline in various part of the brain and therefore be associated with the mood and other mental processes.

There is one single gene for COMT, which codes for both S-COMT and MB-COMT using two separate promoters. Both rat and human S-COMTs contain 221 amino acids, and their molecular weights are 24.8 and 24.4 kD, respectively. Rat MB-COMT contains 43 and human MB-COMT 50 additional amino acids of which 17 (rat) and 20 (man) are hydrophobic membrane anchors. The rest of the MB-COMT molecule is suspended on the cytoplasmic side of the intracellular membranes. Rat S-COMT has been recently crystallized at 1.7 - 2.0 Å resolution. The active site of COMT consists of the AdoMet-binding domain and the actual catalytic site. The catalytic site is formed by a few amino acids that are important for the binding of the substrate, water and Mg^{++}, and for the catalysis of O-methylation. The Mg^{++}, which is bound to COMT only after AdoMet binding, improves the ionization of the hydroxyl groups. The lysine residue (Lys144) which accepts the proton of one of the hydroxyls, acts as a general catalytic base in the nucleophilic methyl transfer reaction.

COMT knock-out mice seem to breed and develop quite normally but there are some gender-dependent behavioural changes pointing to the importance of the brain COMT activity in the normal behaviour. Lack of COMT seems to abolish the normal response to sodium loading.

A series of new and highly selective COMT inhibitors have been developed. Entacapone, nitecapone and tolcapone are nitrocatechol-type potent COMT inhibitors *in vitro* (K_i in nanomolar range) whereas CGP 28014 is a hydroxypyridine derivative and ineffective *in vitro*. In animal studies, these compounds inhibit effectively the O-methylation of L-dopa, thus improving its bioavailability and brain penetration and potentiating its behavioural effects. Entacapone and nitecapone have mainly a peripheral effect whereas tolcapone and CGP 28014 inhibit the O-methylation also in the brain. In human volunteers, both entacapone, nitecapone and tolcapone inhibit dose-dependently the COMT activity of erythrocytes, and improve the bioavailability of L-dopa and inhibit the formation of 3-OMD.

In clinical studies in PD patients, both entacapone and tolcapone potentiate the therapeutic effect of L-dopa and prolong the daily ON time by 1 - 2 h. In the clinic, COMT inhibitors have been well tolerated, and the number of premature terminations has been low. In general, the incidence of adverse events has been higher in tolcapone-treated patients than in entacapone-treated patients. The main events have comprised of dopaminergic and gastrointestinal problems. Tolcapone has been associated with diarrhoea in about 16 to 18% of cases and entacapone in less than 10% of cases. Diarrhoea has led to discontinuation in 5 - 6% of patients treated with tolcapone and in 2.5% of those treated with entacapone. Urine discolouration to dark yellow or orange is related to the colour of COMT inhibitors and their metabolites. Elevated liver transaminase levels are reported in 1 to 3% of patients treated with tolcapone but very rarely, if at all, in patients treated with entacapone. Some cases of acute, fatal fulminant hepatitis have been described in association of tolcapone when more than 100 000 patients have been treated. Therefore tolcapone marketing was suspended in Europe and Canada. No restrictions of the use of entacapone have been proposed.

For safety reasons it would be necessary to clarify the new directions of the metabolism of L-dopa in case when its metabolism by both dopa decarboxylase and COMT is inhibited. Also, the consequences of inhibition of the inactivation of catecholestrogens by COMT inhibitors should be studied in detail.

P.T. Männistö & S. Kaakkola: Catechol-O-methyltransferase (COMT): Biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol. Rev. 199:51:593-628.

DISTRIBUTION OF CATECHOL-O-METHYLTRANSFERASE

Ilkka Reenilä
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Catechol-O-methyltransferase (COMT) is found in almost all mammalian tissues. COMT metabolizes molecules which contain a catechol group, such as xenobiotics, drugs (e.g. L-DOPA) and catecholestrogens. The two isoforms, membrane-bound (MB-) and soluble (S-) COMT differ in their subcellular distribution and they have different enzyme kinetic properties. MB-COMT has a lower reaction capacity but higher affinity towards the substrates. Thus, at lower (physiological) concentrations of the substrates, MB-COMT is supposed to be more important than S-COMT.

Highest COMT enzyme activity is found in liver which metabolize the circulating catechols and, in addition to gastrointestinal COMT, attenuate the entry of ingested catechols. Kidney has a second highest COMT activity. COMT inhibitors cause a natriuretic effect which may be due to inhibition of dopamine metabolism in the kidney. In the brain COMT locates mainly in the astroglial cells surrounding the synapse. However, also some COMT is present in the postsynaptic neurons. The basal COMT activity in tissues is rather stable. However, infusion of toxic substances in the brain increase COMT activity which could be found within a few days in the microglial cells and later in proliferating astroglial cells. In some other tissues, e.g. in human breast cancer, the amount of COMT is changed. This is related to the metabolism of catecholestrogens.

In general, by ubiquitous distribution of COMT the tissues are protected from high amounts of endogenous catecholamine transmitters and catecholestrogens, but also from exogenous catechol-derived substances.

INHIBITION OF COMT BY SECOND-GENERATION COMT INHIBITORS: IN VITRO AND EX VIVO STUDIES

Markus M Forsberg, Pekka T Männistö
Department of Pharmacology and Toxicology, University of Kuopio, Finland

The second-generation catechol-O-methyltransferase (COMT) inhibitors, entacapone and tolcapone, have recently been introduced as adjuncts to levodopa-carbidopa therapy of Parkinson's disease. In the present study the inhibitory effect of entacapone on COMT was compared with that of tolcapone in vitro and ex vivo in order to elucidate the differences in potency and duration of action of these drugs. Also the effects of aqueous solubility and dissolution of entacapone on its absorption was studied in rats.

In vitro studies. The IC50 values of entacapone, tolcapone and nitecapone (not in clinical use) for rat liver COMT did not differ significantly. However, usually tolcapone but occasionally entacapone has been reported to be most potent of COMT inhibitors. Different experimental conditions may explain this partly.

Ex vivo studies. Both entacapone and tolcapone produced strong inhibition of COMT in rat peripheral tissues (duodenum, kidney, liver) at a dose 10 mg/kg (p.o.). Duodenal COMT activity was completely inhibited half an hour after drug administration. However, entacapone inhibited striatal COMT activity only by 34 % and tolcapone by 45 %. In entacapone-treated animals COMT activity was recovered within 6 h, whereas in tolcapone-treated animals inhibition was still present in striatum and kidney at 8 h. In conclusion, tolcapone was more potent inhibitor and had longer duration of action. These results are consistent with previous findings obtained from separate studies.

Absorption studies. Oral administration of entacapone suspension (pH 3.0) produced moderate inhibiton of COMT in red blood cells (RBC), but administration of entacapone as a buffer (pH 7.4) or as a hydroxypropyl-b-cyclodextrin solution (pH 3.0) produced significantly stronger inhibition of COMT in RBC. In conclusion, aqueous solubility of entacapone seems to be inadequate in GI tract so that optimal absorption and pharmacodynamic effect could be reached.

EFFECT OF NMDA/GLYCINE SITE ANTAGONIST L-701,324 ON COCAINE-STIMULATED DOPAMINE RELEASE IN THE RAT NUCLEUS ACCUMBENS

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[(1)]Department of Pharmacology, University of Tartu; [(2)]Department of Clinical Neuroscience, Division of Alcohol and Drug Dependence Research, Karolinska Institutet

Antagonists at the NMDA/glycine site have been shown to significantly attenuate some behavioural effects of addictive drugs including cocaine-induced behavioural sensitization as well as the place preference induced by amphetamine and morphine. In the present study we investigated the role of NMDA/glycine site in the pharmacodynamics of cocaine with regard to its effect on dopamine release in the nucleus accumbens. Microdialysis was conducted in freely moving male Wistar rats. The NMDA/glycine site antagonist L-701,324 was administered 2, 4, or 8 mg/kg IP 40 min prior to cocaine (15 or 30 mg/kg IP). As expected, cocaine brought about a significant increase in the accumbal dopamine release. None of the doses of L-701,324 had any significant influence on this effect, neither did L-701,324 alone reliably affect the levels of accumbal dopamine. Our results indicate that a systemically administered NMDA/glycine site antagonist fails to alter the dopamine release in the nucleus accumbens by itself, neither does it reliably modify the effect of cocaine.

EFFECTS OF μ-OPIOID RECEPTOR AGONISTS ON NIGROSTRIATAL DOPAMINE SYSTEM OF ALCOHOL-PREFERRING AA AND ALCOHOL-AVOIDING ANA RATS

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Previous studies revealed that alcohol-preferring AA rats consume aqueous solution of etonitazene (μ-opioid receptor agonist) more than alcohol-avoiding ANA rats. Furthermore, μ-agonist morphine induces a larger locomotor stimulation in the AA rats than in the ANA rats. We have now studied the roles of nigrostriatal and mesolimbic dopaminergic pathways in the differential behavioural responses of AA and ANA rats to μ-receptor agonists. Acute morphine increased striatal dopamine metabolism and dopamine release (3-MT level) more in the AA than ANA rats. No differences were found in the nucleus accumbens or olfactory tubercle, projection areas of the mesolimbic pathway. Density of μ-receptors was measured with a quantitative autoradiography. Bindings of mu-antagonist [^{3}H]CTOP and μ-agonist [^{3}H]DAMGO were significantly higher in the substantia nigra and striatal patches in the AA rats than in the ANA rats. Function of μ-receptor system of these rat lines was compared by using a DAMGO-stimulated [^{35}S]-GTP-gamma-S binding in the brain sections. DAMGO-induced binding of [^{35}S]-GTP-gamma-S was greater in the substantia nigra and striatal patches of the AA rats than ANA rats. The data indicate that the μ-opioid receptor agonists activate nigrostriatal dopamine system more in the alcohol-preferring rats than alcohol-avoiding rats. Strong activation of this pathway in the AA rats is associated high density of μ-receptor in the nigrostriatal system as compared with the ANA rats. These results suggest that differences in the behavioural effects of μ-opioids between these rat lines are at least partially due to differential activation of nigrostriatal system by μ-opioid receptor agonists between the AA and the ANA rats.

EFFECTS OF REPEATED ADMINISTRATION OF COCAINE AND MORPHINE ON NIGROSTRIATAL AND MESOLIMBIC DOPAMINE IN AA AND ANA RATS

Janne AV Mikkola[(1)], **Honkanen A**[(1)], **Piepponen TP**[(1)], **Kiianmaa K**[(2)], **Ahtee L**[(1)]

[(1)]Department of Pharmacy, Division of Pharmacology and Toxicology, University of Helsinki, Finland; [(2)]National Public Health Institute, Helsinki, Finland

The cerebral dopaminergic mechanisms were studied in the nucleus accumbens and caudate-putamen of AA and ANA rats after four-day repeated morphine or cocaine treatment. Morphine (1 or 3 mg/kg), cocaine (5 or 10 mg/kg) or saline was administered once daily and the extracellular concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured in freely moving rats by *in vivo* microdialysis on days 1 and 4. Morphine increased accumbal DA, DOPAC and HVA similarly in rats of both lines, and no sensitization of DA release or metabolism was seen in rats of either line given morphine repeatedly. In the caudate-putamen, morphine increased DA, DOPAC and HVA significantly only in the AA rats. During repeated treatment, the morphine-induced elevation of DA metabolites, but not that of DA, was enhanced similarly in rats of both lines. The first administration of cocaine increased DA concentration similarly in rats of both lines in the nucleus accumbens as well as in the caudate-putamen. On the fourth day, the effect of cocaine on accumbal DA was significantly enhanced in the AA, but not in the ANA rats, whereas no such enhanced effect of cocaine was found in the caudate-putamen of either AA or ANA rats. These findings do not support the critical role of accumbal dopaminergic systems in morphine-induced behavioural sensitization. However, the results suggest that the mesolimbic DA release is more readily sensitized to cocaine in the AA than in the ANA rats, which might explain our previous findings that the AA rats are more susceptible to psychomotor sensitization than the ANA rats.

ANTIDEPRESSANTS AND SEROTONIN RECEPTORS

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Serotonin (5-HT) and the 5-HT receptors are proposed to play an important role in the neurobiology of affective disorders. There are data that serotoninergic system, especially 5-HT_2, 5-HT_3 and 5-HT_4 receptors are participating in pathogenesis of several behavioural disturbances and anxiety states. Antagonists of these receptors are used as antianxiety drugs, atypical neuroleptics, and even as antidepressants. 5-HT_{1A} receptor partial agonists buspirone, ipsapirone a. oth. are also used as anxiolytics. We have studied the effects of 5-HT_1, 5-HT_2 and 5-HT_3 agonists and antagonists and their combinations with antidepressants (desipramine, citalopram, fluoxetine) in forced swimming test (FST) and exploratory behaviour tests (open field, elevated plus-maze) in male Wistar rats. It was found that both noradrenergic antidepressant desipramine and serotoninergics citalopram and fluoxetine inhibited exploratory behaviour, exerted anxiogenic-like activity. Similar effects exerted 5-HT_{1A} antagonist WAY-100635 and 5-HT_2 and 5-HT_3 agonists DOI and m-CPBG respectively. These compounds shortened also the passive period (immobility) in FST. Agonists of 5-HT_{1A} receptors buspirone and 8-OH-DPAT prolonged the immobility time in FST and increased exploratory activity in open field test. 5-HT_{1A} agonists also antagonized the inhibitory action (anxiogenic-like effect) of antidepressants on exploratory activity. 5-HT_{1A} antagonist WAY 100635 potentiated the anxiogenic-like action of antidepressants. The 5-HT_{1A} receptor antagonist WAY 100635, like antidepressants, shortened the time of immobility in FST and potentiated the acute effect of antidepressants in this test and also in open-field experiments. Thus, the outcome of the spectrum of behavioral phenomena of antidepressants depends on responses of different subtypes of 5-HT receptors to the release or lack of neurotransmitter in synapses.

APOMORPHINE-INDUCED AGGRESSIVE BEHAVIOUR IN ADULT MALE WISTAR RATS: IMPLICATION TO MONOAMINERGIC NEUROTRANSMISSION

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Apomorphine, an unselective dopamine receptor agonist, induces in laboratory rodents different behavioural phenomena such as hyperlocomotion, stereotyped, and aggressive behaviour. We studied the post mortem monoamine content in four brain regions (frontal cortex, striatum, hippocampus, and hypothalamus) of apomorphine-aggressive adult male Wistar rats. Secondly, the effect of various drugs acting at monoamine receptors and/or reuptake was studied. In all experiments, the repeated apomorphine treatment (1.0 mg/kg, once daily during two weeks) gradually induced aggressive behaviour in the majority of the male Wistar rats. We found only moderate increase of the dopamine metabolism, while there were considerable differences between the animals. Drugs that activate the catecholaminergic neurotransmission intensified the aggressiveness, while the noradrenaline and dopamine receptors antagonists had an opposite effect. The selective serotonin reuptake inhibitors were ineffective, however, a moderate antiaggressive effect was found in animals with increased CNS serotonin content. The drugs acting at serotonin receptor subtypes had no unidirectional effect – their effect depended on the dose used and the receptor subtype specificity. Our experiments demonstrate that the repeated apomorphine administration induces in adult male aggressive Wistar rats a moderate increase of post mortem DOPAC and HVA contents with concomitant decrease of dopamine content. The magnitude of this effect is individual and is dependent on the time point of decapitation after the last apomorphine injection. The drugs with catecholamine-positive profile intensify the apomorphine-aggressiveness, catecholamine receptor blockers attenuate this phenomenon. The effect of drugs acting at serotonin receptors depends on the dose used and the receptor subtype specificity.

SUBTYPE SELECTIVE ALPHA-2-ADRENOCEPTOR AGENTS; CAN WE PREDICT THEIR EFFECTS?

Ewen MacDonald
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More than ten years have elapsed since the cloning and characterization of the three alpha-2-adrenoceptor subtypes, but still today there is a dearth of specific subtype selective agonists and antagonists. The drugs which are specific at alpha-2-receptors (e.g. dexmedetomidine, DEX; atipamezole, ATI) show little or no subtype selectivity. The drugs which do differentiate between subtypes tend to be non-specific (e.g. oxymetazoline, prazosin, chlorpromazine). However, recently genetically manipulated strains of mice have become available which either lack one of these receptor subtypes (so-called knock-outs, ko) or in the case of alpha-2C, also mice over-expressing (oe) this subtype have been created.

The first studies with these mice strains concentrated on the modifications in the cardiovascular responses to non-subtype selective agonists, indicating that the alpha2A receptor was responsible for bradycardia and reduction in blood pressure normally seen after DEX. The alpha-2B-subtype mediated the initial increase in blood pressure seen after i.v. DEX. The alpha-2C-subtype did not seem to be involved in modulation of blood pressure. Later work has shown that the alpha-2C-receptor mediates some inhibitory processes in the brain (perhaps in conjunction with the alpha-2A subtype).

These mice strains allow us to speculate on what a subtype selective agonist would be like. It seems as if an agonist which only activated alpha-2A receptors would resemble DEX (except that it would not cause an initial rise in blood pressure after i.v. administration). An alpha-2B selective compound would constrict those blood vessels and elevate blood pressure. Unfortunately, the studies with the geneticaly manipulated mouse strains would not permit us to predict what an alpha-2C compound would do, perhaps this will only become clear when/if such compounds are eventually synthesized.

DERAMCICLANE –PRECLINICAL EVIDENCE OF ANXIOLYTIC ACTIVITY

Aavo Lang
Department of Physiology, University of Tartu

Deramciclane fumarate (previously EGIS-3886) is a novel anxiolytic compound with high antagonistc affinity at 5-HT2A and 5-HT2C receptors. Our studies performed in the beginning of 1990ies and were among the first ones indicating the anxiolytic activity of deramciclane. The current presentation is based on these studies and on the data of other groups. Deramciclane has a clear anxiolytic activity in social interaction test, punished drinking test (Vogel's test), marble burying behaviour. Deramciclane antagonises anxiogenic effect of caerulein in elevated plus-maze test and m-CPP in light-dark situation test. The locomotion suppression doses of deramciclane are higher (>10 mg/kg) than anxiolytic doses (<10 mg/kg). Deramciclane was tested also in depression models, here it was effective in learned helplessness studies. Deramciclane had effect neither in forced swimming test nor tetrabenazine-induced ptosis even in high doses.Studies on deramciclane have reached the stage of human studies. According to latest results, deramciclane penetrates well the blood-brain barrier in humans and binds to 5-HT2A and 5-HT2C receptors. Despite of these listed successful experimental data, a long way is to go for deramciclane to be proven as clinically effective anxiolytic drug.

THE EFFECTS OF CHRONIC NICOTINE ON BRAIN MONOAMINE SYSTEMS: A UNIQUE WINDOW ON THEIR ROLE IN THE NEUROBIOLOGY OF DEPENDENCE?

David Balfour
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Ninewells Hospital, Dundee, Scotland, UK.

It is now widely accepted that people smoke tobacco in order to enjoy the psychopharmacological properties of nicotine and that many habitual smokers find it difficult to quit the habit because they develop dependence to nicotine. This presentation will focus on the evidence that both acute and chronic nicotine exerts effects on the dopamine projections to the nucleus accumbens which are similar to those of other psychostimulant drugs of abuse. However, unlike amphetamine and cocaine which act on the presynaptic dopamine transporter, the effects of nicotine depend upon its ability to influence impulse flow to the terminal field. As a result, studies with the drug have the potential to shed new light on the neurobiology underlying drug dependence. Dialysis studies show that acute nicotine preferentially increases dopamine overflow in the shell of the accumbens. However, the principal effect of repeated nicotine administration is to cause a selective sensitisation of the neurones which project to the core of the accumbens. The presentation will explore the possibility that the primary consequence of this is to cause a marked increase in extra-synaptic dopamine and that may play a pivotal role in the development of dependence. The presentation will also consider the hypothesis that stimulation of mesolimbic DA neurones protects smokers from other changes in the brain, such as reduced serotonergic activity in the hippocampus, that occur as a consequence of chronic exposure to the drug. It will be proposed that the psychopharmacological consequences of these changes are revealed following nicotine withdrawal and that they contribute significantly to the nicotine abstinence syndrome.

COMPARISON OF THE EFFECTS OF EPIBATIDINE AND NICOTINE ON THE OUTPUT OF DOPAMINE IN THE DORSAL AND VENTRAL STRIATUM OF FREELY-MOVING RATS

Tiina Seppä, Liisa Ahtee
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Epibatidine is a potent nicotinic acetylcholine receptor (nAChR) agonist characterised by remarkable analgesic properties. It has been reported to be 150-fold more potent in stimulating dopamine release from rat striatal slices than nicotine (Sullivan et al., J Pharm Exp Ther 271: 624-631; 1994). However, epibatidine appears not to have reinforcing effects according to a recent self-administration study (Rasmussen and Swedberg, Pharmacol Biochem Behav 60: 567-573; 1998). Therefore, the purpose of the present study was to characterise the interaction of epibatidine with the nigrostriatal and mesolimbic dopamine systems. The effects of the nicotinic acetylcholine receptor (nAChR) agonist epibatidine on the extracellular concentrations of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the dorsal (caudate-putamen) and the ventral striatum (nucleus accumbens) of freely-moving male Wistar rats were studied by in vivo microdialysis. In the dorsal striatum, epibatidine (3.0 µg/kg s.c.) significantly elevated the extracellular concentrations of DA, DOPAC and HVA. In contrast, epibatidine did not alter the extracellular DA in the ventral striatum, but elevated significantly the concentration of DOPAC and also tended to elevate that of HVA. In parallel experiments, nicotine (0.5 mg/kg s.c.) significantly increased DA output in the ventral striatum whereas only a modest and non-significant increase of extracellular DA concentration in the dorsal striatum was found. Earlier studies have shown that the doses of epibatidine and nicotine used in the present study are about equieffective at least with respect to the analgesia-producing or hypothermic effects of the drugs. Comparison of the effects of epibatidine and nicotine suggests that the responses of the mesolimbic and nigrostriatal dopaminergic systems to the two nicotinic receptor agonists differ. According to the present study, epibatidine, in contrast to nicotine, preferentially stimulates nigrostriatal vs. mesolimbic dopaminergic system. Therefore, novel nAChR ligands structurally related to epibatidine may have low abuse potential.

BRAIN MONOAMINES AND LOCOMOTOR ACTIVITY IN MICE DURING CHRONIC ORAL NICOTINE ADMINISTRATION

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There is plenty of evidence for nicotinergic regulation of brain monoaminergic systems. The effects of nicotine on dopamine (DA) systems are interesting because of the important role of the mesolimbic DA pathway in reinforcement and drug dependence. We have earlier shown that nicotine can be administered for 7 weeks in the drinking water to mice in doses resulting in plasma nicotine concentrations similar to those reported in smokers (Pekonen et al. 1993, Eur J Pharmaceut Sci. 1,13-18). By using this route of administration, the mice obtain nicotine mainly during their active time, as smokers usually do. Plasma and brain concentrations of nicotine were at their highest in the dark period, when rodents are active. During the chronic nicotine administration the striatal concentrations of DA metabolites were elevated during the light period in the forenoon. The mice drinking nicotine solution were more active than control mice drinking tap water in the forenoon and for a few hours in the latter part of the dark period. However, chronic nicotine administration did not influence the daily rhythmicity of locomotor activity. The differences in locomotor activity between nicotine-treated and control mice correlated with the differences in striatal concentrations of DA metabolites. Our findings suggest that the mice receive enough nicotine from the drinking water during the chronic experiment to induce changes, which are thought to be involved in nicotine dependence. Further, our results support the suggestions that nicotine's effects on dopamine metabolism are critical for its stimulating and thus, most probably also for its reinforcing effect.

NICOTINIC EFFECTS OF COTININE

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Cotinine is the major metabolite of nicotine. It is slowly eliminated, and in smokers its concentrations are over an order of magnitude higher than those of nicotine. Cotinine has frequently been considered biologically inert despite evidence for its weak pharmacological activity. We have used primary cultures of bovine adrenal chromaffin cells to study effects of cotinine on cell signalling. Cotinine time- and concentration-dependently evokes catecholamine release from the cells. It elevates the intracellular free calcium ion concentration and increases protein kinase C activity. These nicotine-like effects of cotinine are seen only at high concentrations but they are reversed by nicotinic acetylcholine receptor antagonists and by removing extracellular Ca^{++}. The cotinine-evoked increase in intracellular [Ca^{++}] is only in part inhibited by Ca^{++} channel blocker nimodipine and not affected by Na^{+} channel blocker tetrodotoxin or by emptying the endoplasmic reticular Ca^{++} stores by thapsigargin. Cotinine pre-exposure concentration-dependently inhibits nicotine-induced Ca^{++} transients, protein kinase C activation and catecholamine release, but it does not reduce the responses to depolarising [K^{+}] or to Na^{+} channel opener veratrine. Furthermore, nicotine pretreatment reduces cotinine-evoked Ca^{++} transients. Thus, cotinine functionally behaves as a low-affinity nicotinic agonist at chromaffin cell receptors. Cotinine displaces the nicotinic receptor ligand [^{3}H]epibatidine from chromaffin cell membrane binding sites in a one-site fashion. Nevertheless, the affinity of cotinine for the labelled receptors is 200 times lower than that of nicotine. Cotinine is unlikely to stimulate the nicotinic acetylcholine receptors, but, in chronic nicotine consumers, it may modify responsiveness to its parent compound.

INFECTION AND CARDIOVASCULAR DISEASE

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Introduction

Atherosclerotic plaques consist of a lipid-rich core covered by a fibrous cap. Early atherosclerosis features endothelial dysfunction, inflammatory cell migration and vascular smooth muscle proliferation. According to the current hypothesis atherosclerosis develops as an inflammatory response to injury of various origin such as oxidized LDL and/or infectious agents. Among the latter the intracellular gram-negative bacterium, *Chlamydia pneumoniae* (*C. pneumoniae*) has been increasingly implicated as a contributing factor to atheroscleros. The main arguments for the chlamydial genesis include: 1) Association of *C. pneumoniae* antibodies (mostly IgA) with atherosclerosis, 2) Detection of *C. pneumoniae* and its components in atheromas, 3) Presence of *C. pneumoniae*, in particular strain AR 39 in endothelium, smooth muscle cells and macrophages of arterial wall with atherosclerosis but not in normal arteries, 4) Favorable effect of macrolide treatment in coronary heart disease and 5) Atherosclerotic changes in animals after experimental exposure to chlamydia and prevention of those by means of macrolide therapy. Infection related pathophysiological mechanisms include: 1) Endothelial dysfunction, 2) Increased expression of vascular adhesion molecules, 3) Increase in LDL and triglycerides and decrease in HDL), 4) Smooth muscle cell proliferation and 5) Increase in platelet and WBC adhesion and 6) Increase in fibrinogen.

C pneumoniae infection impairs endothelial function

ApoE -knockout mice were repeatedly exposed to *C. pneumoniae* to find out whether acute infection would alter endothelial function. After 2 and 6 weeks all infected animals showed increased antibody titers to *C. pneumoniae* but no morphological changes in the aorta or coronary arteries. In the infected group cholinergic vasorelaxation was significantly impaired as compared to the sham-infected group. COX-antagonist, diclofenac improved endothelial function in the presence of the nitric oxide synthase antagonist, L-NAME indicating the production of vasoconstrictive COX-products. This study is the first to demonstrate infection-associated endothelium dysfunction in the absence of overt atherosclerosis.

DEMONSTRATION OF ENDOTHELIN ET_B RECEPTORS IN THE NERVES INNERVATING BOVINE PENILE SMOOTH MUSCLE

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Preliminary pharmacological experiments have suggested that in the bovine retractor penis muscle (BRP) there are relaxation-mediating endothelin ET_B receptors, at least part of which are located on the inhibitory nitrergic nerves. This hypothesis was tested by means of receptor autoradiography and additional pharmacological experiments. Besides the BRP, the bovine penile artery (BPA) was included in the study because these two tissues have an identical innervation, which is very similar to or identical with that of penile smooth muscle in many other mammals. Furthermore, the bovine dorsal metatarsal artery (BMA) was used as a reference because its innervation differs in several respects from that of the BPA.

In the BRP, the BPA and the BMA specific binding of the ET_B receptor-selective agonist [^{125}I]BQ-3020 took place predominantly to nerve trunks and minor nerve branches. In the BRP there was also a small amount of specific binding to smooth muscle. But no specific endothelial binding was observed in any of the tissues examined.

The pharmacological studies confirmed that the relaxation of the BRP induced by the ET_B receptor-selective agonist sarafotoxin S6c is susceptible to tetrodotoxin as well as to inhibition of nitric oxide synthase. The relaxation was also characterized by inconsistency, weakness and tachyphylaxis. The electrical field stimulation-induced submaximal relaxation of the BRP was unaffected by stimulation or blockade of ET_B receptors.

The autoradiography suggests that in all the three bovine tissues studied there are ET_B receptors located on nerves independently of the type of efferent nerve. The pharmacological experiments do not support the concept that in the BRP neuronal ET_B receptors exert important immediate effects on the functioning of the penile erection-mediating nitrergic nerves.

NEW STRATEGIES IN THE TREATMENT OF NEURODEGENERATIVE DISORDERS

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Increasing evidence suggests that at least some of the biochemical and morphological correlates of apoptosis or programmed cell death can contribute to the neuronal loss occurring in the neurodegenerative diseases. The better understanding of the molecular events which set the cell to die will offer new therapeutic strategies for the treatment of the acute and chronic neurodegenerative diseases. In our experiments on the primary cultures of the cerebellar granule cells exposed to the oxygen-glucose deprivation (OGD; model of ishemic brain damage) the neuronal death occurred not only via necrosis but also considerable number of dying cells demonstrated the morphological features of apoptosis. Further analysis, however, demonstrated that some important signs of apoptosis like caspase-3 activation cannot be detected in dying neurones. We suggest that in this model of neurotoxicity, the neuronal death is more complex and may include the elements of both apoptosis and necrosis. Next, we tested a number of compounds which we believed could be able to prevent the OGD-induced neuronal damage. Experiments demonstrated that metabotropic glutamate receptor agonist, (1S,3R)-1-aminocycloheptane-trans-1,3-dicarboxylic acid (tACPD), acting at group I and II receptors and selective group II receptor agonist (2S,1'R,2'R,3R')-2(2,3-dicarboxycyclopropyl)glycine DCG-IV) demonstrated neuroprotective action against OGD-induced necrosis as well as apoptosis. Another strategy that could be effective in the treatment of neurodegenerative disorders is based on a possibility of the pharmacological regulation of the neurogenesis. The results obtained in our laboratory demonstrate that generation of the new progenitor cells as well as their differentiation into neuronal or glial phenotypes might be affected by the pharmacological agents. The preliminary experiments demonstrate that compounds like MK-801 or drugs of abuse might affect neurogenesis in vivo.

In conclusion, prevention of the neuronal death and stimulation of the neurogenesis by the pharmacological agents might lead to the discovery of new effective treatments of the neurodegenerative disorders.

NITRIC OXIDE: GOOD, BAD AND UGLY IN BRAIN

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The pathophysiological role of nitric oxide (NO) has been controversial; it is not clear whether NO is foe or friend to brain neurons. In the present study we compared the effects of various NO donors both in vivo andin vitro models. NO and NO donors, such as S-nitrosothiols were not neurotoxic to nigrostriatal dopaminergic neurons when infused to substantia nigra by using stereotaxic apparatus. Neuroprotective properties of S-nitrosoglutathione were studied against iron induced neurotoxicity. Iron infusion caused acute lipid peroxidation in substantia nigra and delayed dopamine depletion in striatum. NO and S-nitrosoglutathione protected against iron-induced lipid peroxidation and neurotoxicity. It is important to note that sodium nitroprusside caused oxidative stress and neurotoxicity with the same dose range as iron. In vitro results demonstrated that NO and S-nitrosothiols suppressed iron induced hydroxyl radical formation and lipid peroxidation. In contrast sodium nitroprusside generated hydroxyl radicals and caused lipid peroxidation in brain homogenates. We further studied the hypothesis that reduced NO, nitroxyl anion might be neurotoxic because it generated hydroxyl radicals in vitro. Intranigral infusion of nitroxyl anion donor, Angeli's salt caused dose-dependent dopamine depletion instriatum. Because nitroxyl anion may be formed in vivo we suggest that situation shifting the balance between NOand its redox forms may convert good NO to ugly nitroxyl anion.

THE EFFECTS OF THE NITRIC OXIDE SYNTHASE INHIBITORS ON THE BEHAVIOUR OF SMALL PLATFORM STRESSED MICE IN THE PLUS-MAZE TEST

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Nitric oxide (NO), an unusual neurotransmitter molecule in the central nervous system, is synthesized on demand nitric oxide synthase from L-arginine. Effects of the NOS inhibitors 7-nitroindazole (7-NI), N^G-nitro-L-arginine (L-NOARG) and N^G-nitro-L-arginine methyl ester (L-NAME) on the behaviour of control and small platform (SP) stressed mice in the plus-maze test were studied. SP stress was induced by placing mice on small platforms (3.5 cm diameter) surrounded by water for 24 h. This model contains several factors of stress like rapid eye movement (REM) sleep deprivation, isolation, immobilization and falling into the water. The plus-maze test was carried out with control and SP stressed mice. SP stress induced an anxiolytic effect that was evidenced by increased percentage of time spent on the open arms of the plus-maze. The administration of NOS inhibitors 7-NI (20.0-120.0 mg/kg) and L-NOARG (20.0 and 40.0 mg/kg) induced an anxiolytic effect and the administration of L-NAME (20.0 and 40.0 mg/kg) – an anxiogenic effect in control mice. In SP stressed mice the effects of NOS inhibitors were changed. Contrary to control mice, 7-NI at a dose of 20.0 mg/kg induced an anxiogenic effect in SP stressed mice and other doses of 7-NI, as well as L-NOARG and L-NAME were without any effect. On the basis of these data we can propose that SP stress induced changes in the function of L-arginine – NOS – NO pathways.

Abstracts of the poster presentations

DHEAS PREVENTS OXYGEN-GLUCOSE DEPRIVATION-INDUCED INJURY IN CEREBELLAR GRANULE CELL CULTURE

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Neurosteroids are synthesized in the central nervous and peripheral nervous system, from cholesterol or steroidal precursors imported from peripheral sources. Neurosteroids are formed primarily by the glia. The pregnenolone and dehydroepiandrosterone and their reduced metabolites can act as allosteric modulators of neurotransmitter receptors, such as GABAA and NMDA and sigma receptors. Dehydroepiandrosterone (DHEA) together with dehydroepiandrosterone sulphate (DHEAS), are the most abundant steroid in the blood of young adult humans. Levels of DHEA(S)in humans decline with the age and during certain types of illness as Alzheimer disease or stress. To test (DHEAS) as a possible neuroprotectant, we studied its effects on the neurodegeneration induced by oxygen-glucose deprivation (OGD) in cultured cerebellar granule cells. DHEAS added to the medium after injury demonstrated neuroprotective effect within the range of concentrations 0.1 - 100 μM. At 10 μM concentration almost full neuroprotection was observed. Similar effect was observed when DHEAS was present only before injury. DHEAS was neuroprotective also against MPP+, colchicine-, glutamate- and NMDA-induced toxicity. Analysis demonstrated that DHEAS eliminated the apoptotic features of the OGD-induced neuronal death: DNA fragmentation and nuclear condensation/fragmentation. Data suggest that DHEAS may have therapeutical potential in the prevention and treatment of ischemic/hypoxic neuronal damage. The neuroprotective action of DHEAS was inhibited by GABAA receptor-linked chloride channel agonist and antagonist, pentoparbital and picrotoxin, respectively. It seems that both GABA-mediated neuronal inhibition and neuronal exication reduce neuroprotective action of DHEAS.

POLY(ADP-RIBOSE)POLYMERASE INHIBITORS: FROM NEUROPROTECTION TO NEUROTOXICITY

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Poly (ADP-ribose) polymerase (PARP) inhibitors have been shown to be protective in conditions associated with oxidative DNA damage like stroke andischemia-reperfusion. However, several lines of evidence suggest that PARP inhibitors may also exert toxic effects.The aim of the present study was therefore to find out whether these contraversial responses to PARP inhibition can be induced in vitro using the primary culture of cerebellar granule cells and to assess the mechanism of PARP inhibition with 3-aminobenzamide in these settings. The results demonstrate that PARP inhibition was toxic in the case of massive necrosis induced by severe oxygen-glucose deprivation (OGD). 3-aminobenzamide-induced toxicity was associated with increase in ss DNA breaks and resulted in caspase-3 activation and internucleosomal DNA cleavage suggesting apoptotical cell death. PARP inhibitor was completely ineffective in the case of colchicine-induced apoptotic cell death, associated with caspase-3 activation, PARP cleavage and internucleosomal DNA fragmentation. At the same time PARP inhibitor was protective against mild OGD that was not associated with caspase 3 activity but with internucleosomal DNA cleavage referring to some kind of intermediate cell death between apoptosis and necrosis. In conclusion the results of this report demonstrate that the effect of PARP inhibition is dependent entirely on the type of cell death - an enhancement of the toxicity in the case of massive necrotic cell death (severe OGD), no effect in the case of apoptotic cell death (modeled by colchicine) and protection in the case of intermediate cell death (mild OGD).

ANTIOXIDATIVE ACTIVITY OF THE NEW DIHYDROPYRIDINE DERIVATIVES

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Three 1,4-dihydropyridine (DHP) derivatives glutapyrone, tauropyrone (amino acid containing DHP) and cerebrocrast (representative of classical DHP structure) were synthesised at the Latvian Institute of Organic Synthesis. In contrast to classical dihydropyridine type calcium channel antagonists, these compounds lack calcium channel antagonistic properties. Since in our previous studies, glutapyrone, tauropyrone and cerebrocrast were found to exert protective activities against a variety of neurotoxic stimuli-induced toxicity in cerebellar granule cells and in PC12 cell line, the aim of the present study was to investigate the possible involvement of antioxidative activity in their protective actions in cerebellar granule cells using 2',7'-dichlorofluorescein diacetate (DCF-DA) assay. One of the tested DHPs, cerebrocrast (0,1 microM), totally prevented the MPP+-induced production of free radicals. Cerebrocrast was less effective (protection by 52,8%) against glutamate triggered oxidative stress. Other tested compounds, glutapyrone and tauropyrone (both 10 microM), decreased only glutamate-induced free radical production by 57,1% and 77,0%, respectively. Obtained results indicate that antioxidative activity plays a great role in the neuroprotective actions of cerebrocrast, glutapyrone and tauropyrone.

A RAPID AND SENSITIVE STEP GRADIENT HPLC ASSAY OF GLUTAMATE, GLYCINE, TAURINE AND GABA WITH AN EMPHASIS ON MICRODIALYSIS SAMPLES

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The analysis of amino acid neurotransmitters from cerebral microdialysates is of increasing interest in the field of neuroscience. We developed a rapid step-gradient HPLC method for determination of glutamate, glycine and taurine, and a separate method for determination of γ-aminobutyric acid (GABA) from striatal microdialysates. The amino acids were pre-column derivatized with o-phthalaldehyde/2-mercaptoethanol by using automatic refrigerated autoinjector. Separation of the amino acids was established with a non-porous ODS-II HPLC column, late-eluating substances were washed out with one-step low pressure gradient. Concentrations of the amino acids were determined with a fixed wave-length fluorescence detector. The detection limit for GABA was 80 fmol in a 15 µl sample, detection limits for glutamate, glycine and taurine were not determined because their concentrations in striatal perfusates were far above their detection limits. Total analysis time was less than 12 minutes, including the wash-out step. The methods described are relatively simple, sensitive, inexpensive, and fast enough to keep up with the microdialysis sampling.

SEROTONIN 5-HT2A/2C AND 5-HT3 RECEPTORS MEDIATE THE EXPLORATORY BEHAVIOUR AFTER ACUTE ANTIDEPRESSANT ADMINISTRATION IN RATS

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All three major classes of antidepressants, the monoamine oxidase inhibitors (MAOI), the classic tri- and tetracyclic antidepressants (TCA), and the selective serotonin reuptake inhibitors (SSRI) may elicit anxiety and sleep disturbances during the first days of administration. The neurobiological basis of these phenomena is not known. Laboratory tests based on the rodents, exploratory behaviour are widely in use for elucidation of anxiolytic and anxiogenic properties of drugs. In the tests of exploratory behaviour (the elevated plus-maze and open field tests), after acute antidepressant administration an anxiogenic-like effect is found, while after repeated administration this effect disappears or replaces with an anxiolytic-like effect. The aim of the present study was to investigate the role of serotonin 5-$HT_{2A/2C}$ and 5-HT_3 receptors in the open field and elevated plus-maze test after acute antidepressant treatment. Acute desipramine (TCA) and citalopram (SSRI) treatment induced an anxiogenic-like effect in the elevated plus-maze test, the intensity of this effect varied somewhat between the experiments. Similar effect was found in the open field test. The 5-$HT_{2A/2C}$ receptor antagonists ketanserin and ritanserin were ineffective in the elevated plus-maze. These compounds intensified the antiexploratory effect of citalopram treatment. The 5-HT_3 receptor antagonist ondansetron failed to reverse the antidepressant-elicited antiexploratory effect, but showed an anxiolytic-like profile in itself. In conclusion, our experiments confirm that acute antidepressant treatment elicits an anxiogenic-like effect and some elements of this effect may be mediated via the serotonin 5-$HT_{2A/2C}$ and 5-HT_3 receptors.

PROTECTIVE EFFECT OF MELATONIN AND PINOLINE IN MPTP-INDUCED NEURONAL DAMAGE

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In the recent years melatonin has been shown to be a potent antioxidant and free radical scavenger. Antioxidant activity of melatonin has been investigated in different in vitro and in vivo systems. Recently we demonstrated that 6-methoxy-tetrahydro-beta-carboline (pinoline) has antioxidant activity comparable to melatonin in vitro. In the present study we compared the antioxidant activity of melatonin and pinoline in the MPTP-induced neurotoxicity in mice. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a drug which damages dopaminergic neurons by generating of free radicals. Melatonin (10 mg/kg), pinoline (20 mg/kg) or vehicle was administered intraperitoneally to mice 30 min prior to a sc injection of MPTP (20 mg/kg). After MPTP treatment, the animals received melatonin, pinoline or vehicle injections every hour for three hours. Mice were killed 4 hours after the MPTP injection. Significant increases in lipid peroxidation were observed in corpus striatum and hippocampus but not in cerebral cortex. Treatment with melatonin and pinoline completely reversed the lipid peroxidation products. These data confirm the neuroprotective effect of melatonin in MPTP model of Parkinson's disease. Literature data suggest that some beta-carbolines can induce neuronal damage like MPTP; effect of pinoline has not been investigated in this model. Our experiment revealed that unlike several other beta-carbolines, pinoline has neuroprotective effect in MPTP model of neuronal damage.

EFFECT OF THE NEUROPEPTIDE CART ON THE LEVELS OF MONOAMINES, 5HT, AND THEIR METABOLITES IN THE RAT BRAIN

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Cocaine- and amphetamine-regulated transcript (CART) is a brain-located peptide. It has been shown to mediate the anorectic effects of leptin, and furthermore, proposed to be involved in the action of psychomotor stimulants as amphetamine and cocaine. In the present study we examined the influence of a CART peptide fragment, CART 89-103, on the levels of brain monoamines, 5HT, and their metabolites. CART 89-103 was administered to male Wistar rats at 0.05 or 5.0 nmol ICV. We used a sensitive and selective high-performance liquid chromatography method with electrochemical array detector to analyse the tissue homogenates from several brain structures, including the frontal cortex, striatum, hippocampus, hypothalamus, and cerebellum. CART given at 0.5 nmol ICV tended to increase the concentrations of dopamine, DOPAC, HVA, and 5HT in striatum, whereas no reliable changes were found elsewhere. As we consider the results of the present study as the preliminary ones, we propose CART to participate in the regulation of striatal monoaminergic and serotoninergic neurotransmission.

DOPAMINE RELEASE IN NUCLEUS ACCUMBENS AND LOCOMOTOR ACTIVITY AFTER ACUTE AND CHRONIC NICOTINE TREATMENT IN THE ALCOHOL-PREFERRING AA AND ALCOHOL-AVOIDING ANA RATS.

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The aim of the study was to investigate the importance of the interaction between central dopaminergic and cholinergic mechanisms by studying the effects of nicotine on locomotor activity and the release of dopamine in the nucleus accumbens of the alcohol-preferring AA (Alko Alcohol) and alcohol-avoiding ANA (Alko Non-Alcohol) rats with in vivo microdialysis. Nicotine was administered acutely (0.25, 0.50, or 0.75 mg/kg, SC) or repeatedly once daily (0.5 mg/kg, SC) for eight days. Microdialysis samples were collected from freely moving animals every 10 minutes, and concentrations of dopamine and its metabolites were determined in the dialysate with microbore EC-HPLC. An acute dose of nicotine increased locomotor activity and the extracellular levels of dopamine, DOPAC and HVA suggesting stimulation of dopamine release by nicotine. No difference in the stimulation of locomotor activity or dopamine release by nicotine was found between the rat lines. The plasma concentrations of nicotine were also identical. The rats treated repeatedly with nicotine also showed an increase in locomotion and accumbal levels of dopamine and its metabolites. On the challenge day one week after termination of nicotine or saline injections, rats previously treated with nicotine were activated more by nicotine than saline-treated rats. This behavioral sensitization was not accompanied by an increase in the amplitude of the neurochemical response to nicotine , but the duration of the increase of DOPAC was longer in the nicotine than saline-treated rats. The increase of locomotor activity and metabolite levels were, however, similar in both rat lines. These data suggest that differences in the interaction of central dopaminergic and cholinergic mechanisms probably do not contribute to the differences in ethanol consumption between the AA and ANA rats.

EFFECTS OF MORPHINE IN MICE WITH ALTERED AMPA RECEPTOR GluR-A SUBUNITS

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AMPA-type glutamate receptors have been suggested to be involved in the neurobiological mechanisms of drug addiction. We have generated two mouse lines, one lacking functional GluR-A subunits (A-/- knockout line) and another one designed to have impaired calcium permeability of the GluR-A subunit-containing AMPA receptors (arginine R/R-mutants). These lines are healthy, but they show slightly increased locomotor activity and ataxia. Acute morphine administration enhanced locomotor activities of the GluR-A-/- more than those of their

wild-type littermates. Tolerance development to tail-flick antinociception and naloxone-precipitated withdrawal symptoms after treatment with increasing morphine doses were reduced in the A-/- mice as compared to the other lines, although blood and brain morphine levels were similar between the knockouts and littermates. The sensitization of locomotor activity responses to repeated daily morphine was enhanced in the A-/- and R/R mutants only when the sensitization was context dependent, while the wild-type mice showed sensitized responses independent of the treatment regimen. The results indicate that GluRA subunit-containing AMPA receptors are involved in the acute and chronic effects of morphine, and that their functional impairment affects development of morphine dependence sings.

ETHANOL INCREASES NEUROGENESIS IN THE JUVENILE RAT BRAIN

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Exposure to ethanol in utero or during yealy development can produce devastating effects on the developing brain. Administration of ethanol during the brain growth sprout causes profound reduction in the brain weight , which is probably due to the depletion of the neuronal populations. Less is known about the effects of ethanol on the neurons that are generated postnatally. Recent study of Ikonomidou et al. (Science, 2000, vol.287, 1056-101060) demonstrated a massive neuronal apoptosis in the various regions of the juvenile rat brain including hippocampus after single administration of ethanol. In our experiments, we administered ethanol in a dose 3 g/kg to 10 days-old rats and measured the volume of the hippocampal sub-regions: granular cell layer (GCL) and hilus of the dentate gyrus. Unexpectedly despite massive neuronal loss we did not detect any changes in the volume of these sub-regions of hippocampus. This prompted us to study the effects of ethanol on the generation of the new progenitor cells in the proliferative zone of GCL. We performed immunohistochemistry for cell specific markers and the thymidine analog bromodioxyuridine (BrdU), a marker of DNA synthesis that labels proliferating cells and their progeny on the brains of the 10 days-old rats subjected to the ethanol (3 g/kg, two injections) treatment. Exposure to ethanol resulted in a rapid increase in the number of BrdU labelled cells in the GCL of the dentate gyrus. Three weeks after BrdU administration newly generated cells in control animals had neuronal morphology and co-expressed the neuronal marker calbindin. In contrast, a considerable number of the newly generated cells in the brain of ethanol-exposed animals expressed the glial marker GFAP. These results demonstrate that acute ethanol exposure to juvenile rats is able to induce cellular re-organisation in the adult hippocampus.

Meeting Diary

August 24-26, 2000,
3rd Nordic-Baltic Symposium on Molecular Pharmacology of 7TM Receptors
Mauno Koivisto Auditorium, BioCity, Turku, Finland
Info: Ulla-Elina Hurme, Dept. of Pharmacology and Clinical Pharmacology, University of Turku, FIN-20520 Turku
Tel.: +358-2-3337513, fax: +358-2-3337216; e-mail: uehurme@utu.fi
http://www.7tm.utu.fi/

September 09-13, 2000
13th Congress of the European College of Neuropsychopharmacology
Munich, Germany
Info: 13th ECNP Congress, CONGREX HOLLAND BV, P.O. Box 302
1000 AH Amsterdam, The Netherlands
phone: +31 20 50 40 205; fax: +31 20 50 40 225; e-mail: ecnp@congrex.nl
http://www.ecnp.nl/Congresses/2000Munich/

September 10-13, 2000
International Conference on Inflammopharmacology and VII Side-Effects of Anti-Inflammatory Drugs Symposium
Sheffield, England, United Kingdom
Info: Professor KD Rainsford, Biomedical Research Centre, Sheffield Hallam University Howard Street, Sheffield S1 1WB, UK
Tel: (44) (114) 225-2934; Fax (44) (114) 225-2020; e-mail: k.d.rainsford@shu.ac.uk
http://www.shu.ac.uk/schools/sci/biomed/INFLAM2000.html

October 14-18, 2000
New Molecular Targets for Cancer Therapy
St. Petersburg Beach, FL, United States
Info: Continuing Education Office, Moffitt Cancer Center; 12902 Magnolia Drive, FOW-PR/EDU,, Tampa, FL 33612-9497,USA
Tel: +1-813 632-1775, E-mail: seaster@moffitt.usf.edu
http://www.moffitt.usf.edu/promotions/moletar/index.htm

November 09-12, 2000
European Parkinson's Disease Association Conference
Vienna, Austria
Info: Tel: +44-1273-686889; Fax: +44-1273-570082; e-mail: carolyn@martlet.co.uk
http://www.shef.ac.uk/misc/groups/epda/vehome.htm

December 07-09, 2000
4th Conference on Pain Management And Chemical Dependency
Washington, DC, United States
Info: Keegan Young
Tel: +1-770 751-7332; Fax: +1-770 751-7334; E-Mail:meetings@imedex.com

February 11-14, 2001
Steroid Hormones and Nervous System
Torino, Italy
Info: e-mail neurosteroids.2001@unito.it
http://medicina.medfarm.unito.it/dipart/dafml/gcp/info/

June 7-8, 2001
XVI Helsinki University Congress of Drug Research
Helsinki, Finland
Info**:** Outi Salminen, Department of Pharmacy, Division of Pharmacology and Toxicology
P.O.Box 56 (Viikinkaari 5), FIN-00014 University of Helsinki, Finland
Tel: +358-9-19159459, fax 358-9-19159471; e-mail outi.salminen@helsinki.fi
http://www.biocenter.helsinki.fi/drugres/

July 15-19, 2001
International Narcotic Research Conference, INRC-2001
Helsinki, Finland
Info: e-mail eija.kalso@helsinki.fi
http://www.biocenter.helsinki.fi/inrc/inrc2001/index.htm

September 12-15, 2001
5th Congress of the European Association for Clinical Pharmacology and Therapeutics
Odense, Denmark
Info: Professor Kim Brøsen; Institute of Public Health; Clinical Pharmacology
University of Southern Denmark; Winsloewparken 19, DK-5000 Odense C, Denmark
Tel. +45 65 50 37 51; Fax +45 65 91 60 89; E-mail: k-brosen@cekfo.sdu.dk
http://www.ou.dk/med/homepages/eacpt/eacpt5.html

October 21-24, 2001
2nd International Meeting on Antimicrobial Chemotherapy in Clinical Practice (ACCP)
Portofino, Italy
Info: Matteo Bassetti
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http://www.multimedia.it/congress_studio/ACCP/Default.htm

May 18-21, 2002
11th International Conference on Cardiovascular Pharmacotherapy
Montreal, QC, Canada
Info: Tel: +1 514 874 19 98; Fax:+1 514 874 15 80; e-mail:info@iscp2002.com
http//www.iscp2002.com/

July 07-12, 2002
IUPHAR 2002: 14th World Congress of Pharmacology
San Francisco, CA, United States
Info: e-mail iuphar@aspet.org
http://www.iuphar2002.org/

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