disorders, such as depressive symptoms, generalized anxiety and phobias. In conclusion, paroxetine is an effective and well tolerated treatment for the control of panic disorder.

P-46 Paroxetine long-term safety and efficacy in panic disorder and prevention of relapse: A double blind study

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Keywords: Paroxetine; Selective serotonin reuptake inhibitors; Panic disorder; Relapse prevention

This is the only published study to assess the role of selective serotonin reuptake inhibitor (SSRI) in relapse prevention in a double blind fashion. One hundred and thirty-eight responders from a 10-week, double blind, placebo-controlled study in patients with DSM-III-R panic disorder (Dunbar et a1., 1995) were entered into a 6-month extention study to evaluate the long term efficacy and safety in panic disorder. This study comprised two phases. In the maintenance phase (MP), patients continued one current medication for 3 months, while in the randomization phase (RP), responders were rerandomized in a double blind fashion, to either their current treatment or to placebo for a further 3 months. Of the 138 responders who entered MP (30 placebo, 34 paroxetine 10 mg, 34 paroxetine 20 mg, 40 paroxetine 40 mg), 76% (105 patients) continued to RP (62 placebo, 43 paroxetine combined). During MP the efficacy of paroxetine (mean frequency of full panic attacks; mean CGI severity of illness; % with no full panic attacks or >50% decrease in attack frequency) remained unchanged relative to the end of the 10-week study. Thirty percent (11/37) of patients crossing over from paroxetine to placebo relapsed during RP, while only 5% (2/43) of patients continuing on paroxetine treatment relapsed ($p = 0.002$; Chi-square test). The median time to relapse after crossing over from paroxetine to placebo was 14 days and for the 2 patients in the paroxetine group was 14 and 28 days. Paroxetine treatment for up to 6 months demonstrated continuing therapeutic efficacy, and during MP was associated with a generally lower incidence adverse events compared with the initial 10-week study. During RP, the incidence of most common adverse events was not appreciably different between the placebo and combined paroxetine groups. In conclusion, therapeutic efficacy was maintained during long term paroxetine treatment for up to 6 months and importantly, garoxetine was effective in the prevention of relapse. Furthermore, good tolerability for paroxetine with a lower incidence of adverse events compared with the short term study.

Reference

Dunbar, G., Steiner, M., Oakes, R., Gergel, I., Burnham, D., Wheadon, D.E. (1995) A fixed dose study of paroxetine (10 mg, 20 mg, 40 mg) and placebo in the treatment of panic disorders, Eur Neuropsychopharmacol 5, 361.

P-47 The effects of amitriptyline and clomipramine on learning and memory in an elevated plus-maze test in mice

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Keywords: Amitriptyline; Clomipramine; Learning and Memory; Elevated plus-maze

Recent publications suggest that antidepressant drugs, especially those having sedative action and antimuscarinic effect, can exert detrimental effects on human memory (Thomson, P.J. 1991). Amitriptyline, having a high incidence of antimusarinic as well as marked sedative effect, was the most reported drug with regard to memory impairments. On the other hand, clomipramine having animuscarinic activity but causing less sedation, had no measurable effect on learning and memory (Lilequist R. et al., 1974). The present study was undertaken to obtain some evidence for the disruptive effects on learning and memory induced by antidepressant drugs by investigating the effects of amitriptyline and clomipramine on learning and memory in an elevated plus-maze test in mice. This test is suggested to be a simple method for the evaluation of learning and memory in mice (Itoh J. et al. 1990). The elevated plus-maze consists of two open and two closed arms and the principle of the method is based on the assumption that mice prefer the enclosed arms to the open arms.

Male mice, 22-30g, were used throughout the experiment. On day 1 before the first trial, the mice were given intraperitoneally with saline (Control), 10 mg/kg amitriptyline (Amitriptyline) or 10 mg/kg clomipramine (Clomipramine). One hour after the injections, the mice were individually placed at the end of one open arm of the apparatus facing away from the central platform and the time it took for the mouse to move from the open arm to either of the enclosed arm (Transfer latency, TL) was recorded. Twenty-four hours later, the mice were put into the open arm and the TL was recorded again. All results are expressed as the mean of time (second) $\pm$SE. Statistical analysis were carried out by two-tailed Mann-Whitney U-test.

In all groups, TLs on day 2 were found shortened when compared with those on day 1. The differences were significant in the Control (59.8 ± 5.9 to 43.5 ± 7.1, $p<0.05$) and Amittriptyline (74.0 ± 7.6 to 38.7 ± 8.5, $p<0.01$) groups, but not in the Clomipramine group (75.1 ± 6.8 to 51.2 ± 9.4, $p>0.05$). The TLs of the Amitriptyline and Climipramine groups, on the other hand, were not significantly different than those of the Control group on both days.

The results of the present study showed that neither amitriptyline nor clomipramine impaired learning and memory in mice, since that shortening and prolongation of TL on day 2 is taken a parameter for the existence and impairment of learning and memory in mice, respectively. However, before concluding that amitriptyline and clomipramine are ineffective in learning and memory in the elevated plus-maze test in mice, different doses of the drugs must be investigated by this test. On the other hand, further experiments are also needed to clarify whether this test is insensitive to the screening of antidepressant-induced memory impairments.

Reference

Itoh, J., Nabeshima, T. and Kameyama, T. (1990) Utility of an elevated plus-maze for the evaluation of memory in mice: effects of nootropics, scopolamine and electroconvulsive shock, Psychopharmacology 101, 27-33.

Liljequist, R., Linnoila, M. and Mattila, M.J. (1974) Effects of two weeks treatment with chlorimipramine and nortriptyline, alone or in combination with alcohol, on learning and memory, Psychopharmacology 39, 181-186.

Thomson, P.J. (199l) Antidepressants and memory, Hum Psychophannacol 6, 79-90.

P-48 The action of kavain, a kava pyrone prepared from the psychotropic remedy Piper methysticum, on presynaptic neurotransmission

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Keywords: Anxiety, Depression, Kava pyrones, Piper methysticum, sodium channels, Synaptosomes

The intoxicating pepper, *Piper methysticum Forst.*, is a remedy indigenous to the South Pacific islands, employed by the natives against depressive disorders, to alleviate anxiety and to counteract fatigue. Because of its psychotropic action, the Federal Board of Health of Germany proved the drug Piperis methystici rhizoma for the treatment of anxiety syndrome. The 4-methoxy-α-pyrones (kava pyrones) are responsible for the pharmacologic properties of the drug but up to date the mode of molecular action is unknown. In the present investigation, the influence of the natural kava pyrone, (+)-kavain, and its synthetic racemate, ($\pm$)-kavain, on presynaptic neurotransmission was estimated as judged by their action on cytosolic free Na^+ and Ca^{2+} ($[Na^+]_i$, $[Ca^{2+}]_i$) and the release of endogenous glutamate from rat cerebrocortical synaptosomes which were stimulated with veratridine, 4-aminopyridine (4AP), and KCl. $[Na^+]_i$ and $[Ca^{2+}]_i$ were determined fluorometrically employing SBFI and FURA-2, respectively, and glutamate-release, was continuously monitored by an enzyme linked fluorometric assay. (+)-Kavain and ($\pm$)-kavain affected neither basal $[Na^+]_i$ and $[Ca^{2+}]_i$ nor spontaneous glutamate-release of non-stimulated synaptosomes at concentrations up to 400 μmol/l, suggesting that the psychotropic effect is not mediated by an induction of neurotransmitter-release. Stimulation of synaptosomes with 5 μmol/1 veratridine, an activator of voltage-dependent Na^+ channels, provoked an increase in $[Na^+]_i$ and $[Ca^{2+}]_i$ by 63 mmol/l Na^+ and 694 nmol/l Ca^{2+}, resulting in a long lasting release of glutamate (rate: 25.6 pmol/sec*mg protein) under these conditions of permanent depolarization. A pre-application of both enantiomers

suppressed the increase in $[Na^+]_i$, $[Ca^{2+}]_i$ and the rate of glutamate-release with IC_{50} (±SD) values of 71±21, 72±7, 120±37 μmol/l (+)-kavain, and 77±21, 90±14, 92±23 μmol/l (±)-kavain, respectively, indicating a non-stereospecific inhibition of Na^+ channels by both kavains. Similar results were obtained by the addition of the K+ channel blocker 4AP to synaptosomes which reduced membrane potential sufficiently (about 15mV) to activate Na^+ channels. 5 mmol/l 4AP induced an increase in $[Na^+]_i$ and $[Ca^{2+}]_i$ by 9 mmol/l Na^+ and 236 nmol/l Ca^{2+}. Since a pre-application of kavain suppressed the increase of both cations and glutamate-release, the inhibition of Na^+ channels by kavain was confirmed, and a competition of kavain with veratridine for the veratridine-binding site on Na^+ channel could be excluded. KCl-depolarization (40 mmol/1) induced an increase in $[Ca^{2+})_i$ by 251 nmol/l Ca^{2+} without affecting $[Na^+]_i$, leading to a release of glutamate at a rate of 35 pmol/sec*mg protein. A suppression of the KCl-induced enhancement of $[Ca^{2+}]_i$ and glutamate-release to about 90% of control could only be observed at high concentrations of 400 μmol/l kavain, suggesting a low affinity of kavain to voltage-dependent Ca^{2+} channels. Regarding to the velocity of kavain-action, an application of (+)-kavain or (±)-kavain, 100 sec prior to the stimulus, was sufficient to block the veratridine- and 4AP effect completely. If kavain was applied subsequently, e.g. to veratridine, the enhanced $[Na^+]_i$ and $[Ca^{2+})_i$ declined with half lives (±SD) of 48±7 and 29±3 sec, respectively, and glutamate-release ceased.

In conclusion, the data indicate a fast non-stereospecific inhibition of voltage-dependent Na^+ channels by kavain as primary target, whereas voltage-dependent Ca^{2+} channels seems to be less affected.

P-49 Flumazenil exhibits a diazepam like-effect in Electroconvulsive shock-treated rats

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Keywords: Flumazenil; Active avoidance; Electroconvulsive-shock

The general assumption that flumazenil is a competitive antagonist at benzodiazepine receptors can be counteracted with its weak agonist or inverse agonistic properties when used in high doses. Flumazenil and ligands with inverse agonistic activity such as Ro 15-4513 have all been suggested to enhance cognitive performance in various experimental paradigms (Lal H. et al., l989).

As is known, electroconvulsive therapy results both in impaired current learning (anterograde amnesia) and loss of acquired learning (retrograde amnesia); the former comprises impairments in both immediate and delayed recall (Lerer B. et al.). The possible neurochemical basis for electroconvulsive shock-induced memory deficits is still a matter of debate. Therefore, we investigated a possible relationship between the benzodiazepine receptor antagonism and electroconvulsive-shock (ECS), thus, the effects of flumazenil on the memory-impairing activity of conversant electroshock in rats.

Rats were administered one electroconvulsive shock daily for 7 days (ECSx7) and 24 hours after the last ECS-treatment, each rat was given 20 training trials for an increasing doses (1-2mg/kg) of diazepam when compared with vehicle-treated rats. On the other hand, locomotor activity was shown to decrease in a dose-dependent fashion with diazepam. Flumazenil alone did not modify the acquisition performance, but it was found to prevent the learning-memory impairment produced only by the lowest diazepam dose (O.5mg/kg) in groups treated simultaneously with diazepam (0.5, 1, 2mg/kg, i.p.) and flumazenil (10mg/kg).

The present results suggest that flumazenil when administered chronically throughout the whole experimental protocol and analysed both the trend of performance through and at the end of 5 days, was shown not to affect acquisition (or learning) performance but to significantly improve the acquisition performance deficit induced by the lowest 0.5 mg/kg dose of diazepam. The discrepancy of the effects of flumazenil against different doses of diazepam needs to be further elucidated regarding the inverse agonistic patency of high doses of flumazenil but in general the antagonistic effect of flumazenil can be attributed to its receptor antagonistic efficiency.

References

Brogden R.N. and Goa K.L. (1988). Flumazenil. Drugs 35, 448-467.

Lal H., Kumer B. and Forster M.J. (l988). Enhancement of learning and memory in mice by a benzodiazepine antagonist. FASEB J. 2, 2707-2711.

Prather P.L., Forster M. J. and Lal H. (1991). Learning and memory-enhancing effects of Ro 15-4513: a comparison with flumazenil. Neuropharmacology 31(3), 299-306.

P-50 The effects of flumazenil on two-way active avoidance, in diazepam-treated rats

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Keywords: Flumazenil, Shuttle-box avoidance, Locomotor activity, Diazepam

Flumazenil is a specific benzodiazepine receptor antagonist which has been shown to reverse the sedative, muscle-relaxing, anxiolytic and anticonvulsant effects of benzodiazepines (Brogden R.N. and Goa K.L., 1988). Moreover, it is very well known to increase the acquisition performances in various learning and memory tasks (Lal H. et al., 1989) and inverse agonist-like properties of the drug has been attributed for its enhancing learning and memory performance (Prather P.L. et al., 1992). Although, the effects of flumazenil on benzodiazepine induced learning and memory impairments has scarcely been studied. The aim of the present investigation was to determine whether flumazenil (Ro 15- 1788), could enhance learning performance when administered chronically alone and simultaneously with diazepam.

The effects of flumazenil on learning task was investigated in a shuttle-box two-way active avoidance test (a conditioned avoidance test) and on locomotor activity in rats. Flumarenil (5, 10, 20mg/kg, i.p.) and diazepam (0.5, 1, 2mg/kg, i.p.) were tested for their influence on acquisition of a conditioned avoidance response. Each rat was given 20 training trials per day for five consecutive days. Locomotor activity was measured following the first day trial of the protocol.

A significant and progressive decrease with the increasing doses (0.5–1– 2mg/kg) of diazepam when compared with vehicle-treated rats. On the other hand, locomotor activity was shown to decrease in a dose-dependent fashion with diazepam. Flumazenil alone did not modify the acquisition performance, but it was found to prevent the learning-memory impairment produced only by the lowest diazepam dose (0.5mg/kg) in groups treated simultaneously with diazepam (0.5, 1, 2mg/kg, i.p.) and flumazenil (10mg/kg).

The present results suggest that flumazenil when administered chronically throughout the whole experimental protocol and analysed both the trend of performance through and at the end of 5 days, was shown not to affect acquisition (or learning) performance but to significantly improve the acquisition performance deficit induced by the lowest 0.5 mg/kg dose of diazepam. The discrepancy of the effects of flumazenil against different doses of diazepam needs to be further elucidated regarding the inverse agonistic potency of high doses of flumazenil but in general the antagonistic effect of flumazenil can be attributed to its receptor antagonistic efficiency.

References

Brogden R.N. and Goa K.L. (1988). Flumazenil. Drugs 35, 448-467.

Lal H., Kumar B. and Forster M.J. (1988). Enhancement of learning and memory in mice by a benzodiazepine antagonist. FASEB J. 2, 2707-2711.

Prather P.L., Forster M.J. and Lal H. (1991). Learning and memory-enhancing effects of Ro 15-4513: a comparison with flumazenil. Neuropharmacology 31 (3), 299-306.

P-51 Effects of L-Arginine on behavioural symptoms of ethanol withdrawal syndrome in rats

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Keywords: Ethanol; Nitric Oxide; L-Arginine; Withdrawal Syndrome; Rat(s)

Although the signs of ethanol withdrawal syndrome (EWS) have been well described, the mechanisms underlying physical dependence or EWS are poorly understood. Recently, it was reported that alcohol withdrawal syndrome may be affected by nitric oxide (NO) (Adams et al., 1995). In a preliminary study, L-arginine, a NO precursor, was shown to prevent the development of audiogenic seizures in ethanol withdrawn rats (Uzbay et al., 1995). The present study was undertaken to examine whether L-arginine would alter the behavioural aspects of EWS in rats.