Isolichenan, an α -glucan isolated from lichen *Cetrariella islandica*, repairs impaired learning behaviors and facilitates hippocampal synaptic plasticity

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Abstract: The acute effects of isolichenan, a homologous α -glucan (1-3)(1-4) (3:2) isolated from lichen *Cetrariella islandica*, on the hippocampal synaptic plasticity were investigated in rats *in vivo*. Isolichenan (1 mg/kg, intravenous treatment) significantly enhanced short-term potentiation evoked by a subthreshold tetanus, without any effect on the basal synaptic excitability (no tetanus stimulation). No effects on a supra-threshold tetanus-evoked potentiation were found. Isolichenan did not influence learning behaviors of intact mice in passive-avoidance tests and of intact rats in the Morris water maze test. When the learning ability of mice was impaired by 30% ethanol pre-treatment, isolichenan (>100 mg/kg, oral treatment) significantly improved memory acquisition in passive-avoidance tests. Similarly, isolichenan (100 mg/kg, oral treatment) repaired memory-impairment induced by β -amyloid peptide (fraction 25-35) in the Morris water maze test in rats. These results suggest positive effects of isolichenan on spatial memory formation in rodents.

Key words: Isolichenan; *Cetrariella islandica*; hippocampus; learning; synaptic plasticity.

Introduction. Isolichenan is a chemically defined homologous α -glucan isolated from lichen *Cetrariella islandica* (L.) Ach. In traditional European medicine, lichenal extracts were often used for treatment of various diseases. Traditional oriental medicine also recognized and used polyssacharides-containing lichens and fungi.¹⁾ However, the pharmacological bases of polyssacharides' effect have only recently been studied.¹⁾⁻³⁾ We have shown that PC-2, an (1-3)(1-4) (3:2) α -glucan isolated from lichen *Flavoparmelia caperata* (L.) Ach, significantly modulated the electrophysiological model of spatial memory, i.e. the formation of long-term potentiation (LTP).³⁾ The primary structure of isolichenan is comparable to that of PC-2. Therefore, we have decided to test this glucan's efficacy in the

above-mentioned electrophysiological model. Synaptic plasticity in the hippocampal area (especially dentate gyrus (DG) granule cells) is important in the initial storage of certain forms of memory,⁴⁾ and the LTP-like phenomena may be active during the real memory formation.⁵⁾

In the second part, we tested isolichenan efficacy in several behavioral models of memory and learning, concretely in passive-avoidance tests and the Morris water maze test (MWM). MWM in rats heavily depends on the intact hippocampal functions, and it is widely regarded as a behavioral counterpart of LTP.⁶⁾ Although the use of passive avoidance procedures has been questioned from several quarters, they are still very popular in evaluation of memory traces. A few results have shown that also passive avoidance tests could correlate with the hippocampal synaptic plasticity.⁷⁾ Consequently, we have used the passive avoidance as an additional behavioral model of memory formation.

Methods. Materials. Isolichenan, an α -glucan with (1-3)(1-4) linkages in the ratio (3:2), $[\alpha]_D + 255^\circ$ (H₂O), I₂ reaction (+), was isolated from lichen *Cetrariella islandica*.^{8),9)} Final samples were chemically identified by ¹H NMR spectra.

Electrophysiological recordings. Male Wistar

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Cetrariella islandica* (L.) Ash (subsp. *islandica*)*Collected at Porvoo/Kulloo, prov. Uusimaa, Finland, on June 17, 1996.**

Fig. 1. Scheme of isolichenan isolation.

rats (190–210 g) were anaesthetized with a combination of urethane and α -chloralose. They were placed in a stereotaxic frame and prepared unilaterally with 250 μ m stainless recording electrode (insulated except at the 500 μ m cut tip) in the DG. An identical bipolar stimulating electrode was lowered into the angular bundle to activate the medial perforant path fibers.¹⁰⁾ The depths of the electrodes were adjusted to produce maximal field excitatory postsynaptic potentials (positive-going) and population spikes (PS). The test stimulation (diphasic; 80 μ sec, 450–600 μ A) was adjusted in amplitude to produce a 3–5 mV PS. Although the PS does not provide full information embraced in the field potential (only its linear approximation), it highly correlates with the responses of DG granule cells.¹¹⁾ Test pulses were applied at 30 sec intervals. After at least 60 min of basal

recording, one set of high-frequency tetanic stimulation composed of 20 pulses at 60 Hz (sub-threshold tetanus) or 100 pulses at 100 Hz (supra-threshold tetanus) was delivered through the stimulating electrodes. Responses were followed during 60 min. PS amplitudes were expressed as percent change from the pre-tetanic baseline values and averaged across 2 min epochs. Isolichenan (1 mg/kg) was injected into the left femoral vein (i.v.) 15 min before tetanus. In one experimental set, an unpurified (crude) isolichenan that contained additional, chemically undefined particles was applied.

On completion of the electrophysiological experiments, lesions were made at each electrode tip (400 μ A, 20 s) in order to verify their placement. Brains were sliced and examined. The subjects with improperly

Fig. 2. Effect of isolichenan on tetanically-evoked potentiation in the DG of rats. Isolichenan was applied (1 mg/kg, i.v.) 15 min before tetanus (20 pulses at 60 Hz). Time course of changes is compared to saline and crude isolichenan treated rats. Results are expressed as percent differences between basal (100%) and stimulated levels. Means of 6 rats $\pm$ SEM. (* $p < 0.05$ from saline group).

placed electrodes were excluded from analysis. Experiments were done between 8:00 a.m. and 2:00 p.m., in order to avoid impacts of the circadian rhythm. Rectal temperature was monitored by a probe. Data were statistically evaluated by one way ANOVA (post hoc Duncan's multiple range test).

MWM. Rats were trained and tested in the MWM between 8:00 a.m. and 3:00 p.m. in a circular water pool (120 cm in diameter). Prior to the actual trials, all animals were evaluated with regard to their ability to swim (milk-colored water temperature, 20 °C) and to climb a visible platform. Then the pool was arbitrarily divided into 4 regions. A transparent escape platform (8 cm in diameter) was placed in the center of the north quadrant of the pool. Rats were allowed 120 s to locate the platform, according to the numerous extra-maze cues (platform-stay, 30 s). In the case of failure, the rat was forced to swim towards the platform. The one daily trial from random positions continued for 4 consecutive days (Day 5–8). The swimming route of a rat was traced by camera and a computerized behavioral tracing analyzer that provided information about the escape latency, swimming time and swimming speed. Body weight and temperature were monitored daily one hour before behavioral procedures. β -amyloid peptide (fraction 25–35) (15 nmol, i.c.v.) was treated 12 days before testing (Day –7) via a cannulae implanted into the lateral ventricle. Isolichenan was given daily from Day 1 to Day 8 (100, 200 mg/kg, p.o.) 30 min before testing.

Fig. 3. Effect of isolichenan on the β -amyloid peptide (β -AP) (fraction 25–35) induced memory impairment in the Morris water maze test. β -Amyloid peptide was treated daily (15 nmol) by an i.c.v. injection for 1 week. Isolichenan was given in the same time-schedule (100, 200 mg/kg, p.o.). Both substances were administered 30 min before the behavioral procedures. Results are expressed as escape latencies during the 4 days of testing. Means of 10–12 rats $\pm$ SEM. (* $p < 0.05$ from β -amyloid peptide group).

Passive avoidance tests. The use of passive avoidance tests (step through and step down test) was already described in detail.⁷ Male Std-ddY mice (5 week old) were used. The apparatus for the step through test consisted of two separated compartments, one of them illuminated and one dark. On the first day (learning trial), a mouse was put into the bright compartment. When the mouse entered the dark compartment, it suffered an electric shock on its feet (36V, 0.2 mA AC). The time when mouse entered the dark compartment was recorded automatically. On the second day (testing trial) the same procedure was repeated. Step down test followed the above-described test, using the same animals. The chamber of the step down test apparatus was equipped with stainless steel grid floor through which an electric foot shock (50V, 0.2 mA AC) was given. A platform was provided above a part of the floor so that mouse could avoid the shock. On the first day (learning trial), mice were exposed to a 10-min learning trial. The number of mice that did not step down was recorded. On the second day (testing trial), the same test procedures were performed at the same time of day for 3 min. The time until mouse step down to the floor (latency) was recorded. Isolichenan (100–400 mg/kg, p.o.) was applied 30 min before learning. In the experimental set, in which mice were treated with ethanol (30%, 0.1/10 g, p.o.), ethanol was given 20 min

Table I. Effect of isolichenan on the step through latencies in passive avoidance test. Means of 10–12 mice $\pm$ SD

Group	Step through latency [sec]	
	Training	Acquisition
Vehicle	24.7 $\pm$ 26.2	147.6 $\pm$ 54.7
30% ethanol	24.3 $\pm$ 35.1	56.1 $\pm$ 60.5**
30% ethanol + isolichenan (100 mg/kg)	24.0 $\pm$ 25.7	90.0 $\pm$ 76.7 [#]
30% ethanol + isolichenan (200 mg/kg)	19.2 $\pm$ 16.3	86.5 $\pm$ 69.0 [#]
30% ethanol + isolichenan (400 mg/kg)	42.7 $\pm$ 39.1	108.0 $\pm$ 73.5 [#]

**p<0.01 from Vehicle group; [#]p<0.05, [#]#p<0.01 from 30% ethanol group.

Table II. Effect of isolichenan on the step down latencies in passive avoidance test. Means of 10–12 rats $\pm$ SD

Group	Step down latency [sec]	
	Training	Acquisition
Vehicle	4.6 $\pm$ 5.6	68.3 $\pm$ 96.6
30% ethanol	2.5 $\pm$ 2.8	7.5 $\pm$ 5.6**
30% ethanol + isolichenan (100 mg/kg)	2.4 $\pm$ 2.1	7.8 $\pm$ 4.8 [#]
30% ethanol + isolichenan (200 mg/kg)	2.0 $\pm$ 2.5	9.1 $\pm$ 8.3
30% ethanol + isolichenan (400 mg/kg)	7.9 $\pm$ 6.7	24.5 $\pm$ 21.4 [#]

**p<0.01 from Vehicle group; [#]#p<0.01 from 30% ethanol group.

before learning.

Results. Fig. 1 illustrates a chemical isolation of isolichenan. Fig. 2 shows that the tetanus of 20 pulses at 60 Hz evoked a mild, but stable potentiation of PS in saline treated rats. Potentiation reached maximum within 5 min and gradually declined to 115–123% of the pre-tetanic values within 1 h. Intravenous application of isolichenan 15 min before tetanus did not affect the pre-tetanic values, but led to a significant enhancement of tetanically-potentiated PS amplitudes (5–60 min). Crude (unpurified) isolichenan remained without significant effect. Isolichenan had no effect on the post-tetanic potentiation evoked by the supra-threshold tetanus of 100 pulses at 100 Hz (data not shown). Fig. 3 demonstrates that the i.c.v. application of β -amyloid peptide led to dramatic deterioration of rats' performances in the MWM. Isolichenan pretreatment (100 mg/kg) significantly repaired the effect of β -amyloid peptide during the last day of testing. No effects on body-temperature and swimming speed were found (data not shown). Additionally, isolichenan had no effect on the escape latency in rats that were not pre-treated with β -amyloid peptide (intact rats) (data not shown).

Results of the passive avoidance tests are summarized in the Tables I and II. Obviously, the ethanol (30%) pre-treatment caused significant impairment of mice memory acquisition in both tests. Isolichenan (100–400 mg/kg) repaired this deleterious effect in step through test and, with lesser efficacy (400 mg/kg), in step down test. Similarly to the MWM, no effects of the tested α -glucan in intact animals (mice without exposure to ethanol) were found (data not shown).

Discussion. In the present study, we have demonstrated that a single administration of isolichenan (a novel α -glucan isolated from lichen *Cetrariella*

islandica) enhanced tetanically-evoked PS potentiation in the dentate gyrus in rats. Isolichenan had no effect on either saturated potentiation (potentiation evoked by a suprathreshold tetanus) or basic synaptic transmission. These results suggested that isolichenan did not act via non-specific increases in neuronal excitability.

The above-described data are in good agreement with our previous report,³⁾ in which we documented comparable effects of an α -glucan (PC-2) isolated from lichen *Flavoparmelia caperata*. This comparability was expectable, since both glucans were characterized by similar chemical structure.

Interpretation of the above-described plastic synaptic enhancement is complicated by a lack of additional experimental data. Specifically, no detailed information on isolichenan passage through the blood-brain barrier is available. Since isolichenan is a linear, easily metabolized α -glucan, it is possible that a part of isolichenan could gain access to hippocampus directly. As an indirect support for this hypothesis, recent study¹²⁾ showed enhancement of synaptic plasticity in the hippocampus by several mono- and di-saccharides. Yet we found that a structurally related glucan, PC-3 that differs from PC-2 and isolichenan only in the ratio of $\alpha(1-3)(1-4)$ linkages was ineffective in the experiments of hippocampal synaptic plasticity.³⁾ This findings urged us to suggest that the chemical structure of the glucans was related to their hippocampal effects, probably via the hormonal relays as previously reported.

Isolichenan did not enhance memory performance of intact rats in the MWM (a behavioral model that is widely regarded as a counterpart of hippocampal synaptic plasticity). Rodents quickly learn the MWM task, and intact rats give no significantly differential response to the drug which might enhance the conven-

tional performance in the MWM. Therefore, for testing new memory-enhancing compounds, memory-impaired animals are used. Actually, we found that the tested glucan significantly improved memory deterioration caused by the β -amyloid peptide (fraction 25–35). This shows that isolichenan effectively influences the hippocampally-based memory performance also in a behavioral model. Since the increased density of the β -amyloid peptide has been associated with the neurological deterioration found in the Alzheimer's disease,¹³⁾ the efficacy of isolichenan may have interesting pharmacological implications.

Step through and step down tests are learning tasks utilizing an electric shock as a negative reinforcer. Thus, any treatment that alters the pain sensitivity and spontaneous movement may secondarily affect the learning performances. However, in our previous experiments⁷⁾ we showed that 30% ethanol did not change the response threshold compared to a vehicle group. These results indicated that the learning impairment induced by ethanol was a direct action on the learning process *per se*. Importantly, isolichenan itself did not affect spontaneous movement in intact or ethanol-treated animals (data not shown). Consequently, the effects of isolichenan did not result from unspecific changes in motor activity and pain responsiveness. Isolichenan significantly repaired the deleterious effect of the ethanol pre-treatment. We hypothesize that isolichenan intervened in the ethanol action on learning. Functional relationship between the hippocampal synaptic plasticity and passive avoidance tasks is not completely clear and straightforward. Some previous work suggested that ethanol inhibits hippocampal synaptic plasticity in a similar manner as it inhibits learning in passive avoidance tasks.¹⁴⁾ It is possible that isolichenan improved the ethanol-induced memory deficit in passive avoidance tests by antagonizing the synaptic plasticity-suppressing action of ethanol in the hippocampus.¹⁴⁾

Isolichenan effectiveness was four times higher (100 mg/kg versus 400 mg/kg) in step through test, as compared to step down test. The difference might have

reflected different stress exposure in the two tests, or different reactivity to foot shocks.

Taken together, isolichenan was documented as a new substance with a significant positive impact on certain forms of memory deficits evaluated in behavioral tests. Importantly, isolichenan enhanced tetanically-evoked synaptic plasticity in the hippocampus, suggesting that its effects might have been triggered by an action on the hippocampal neurotransmission. Further investigations are required to clarify the mechanisms of isolichenan's effects and its possible therapeutic applications.

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