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REVIEW ARTICLE

Effect of Selected Thallophytic Glucans on Learning Behaviour and Short-term Potentiation

Miro Smriga[1] and Hiroshi Saito[2]*

[1]Ajinomoto Co., Inc., Central Research Laboratories, Kawasaki 210-8681, Japan
[2]Department of Pharmacology, College of Pharmacy, Nihon University, Chiba 274-8555, Japan

This paper reviews the effects of thallophytic glucans on rodent cognitive performance modelled by a combination of behavioural and electrophysiological approaches. Glucans were isolated from thallophytic plants, based on prescriptions used in traditional Chinese and Japanese medicine. In parallel with the already described enhancement of hippocampal synaptic plasticity by disaccharides, polysaccharides isolated from lichens *Flavoparmelia caperata* and *Cetrariella islandica*, enhanced hippocampal plasticity and behavioural performance in rats. Copyright © 2000 John Wiley & Sons, Ltd.

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EXPERIMENTAL FRAMEWORK FOR DEVELOPMENT OF PHARMACOLOGICAL MODULATORS OF COGNITION

The number of people suffering from the age-related cognitive disorders, such as senile amnesia, is growing worldwide. However, pharmacological targeting of senile cognitive impairment remains controversial. Since cognition itself is a disputed phenomenon, its experimental representations include a wide range of perspectives. During the past 10 years, we have developed a broad experimental framework for evaluation of centrally active compounds (Saito *et al.*, 1996). We have used three major paradigms: (1) primary neuronal culture for determination of neurotrophic effects of the candidate compounds, (2) electrophysiological evaluation of activity-dependent synaptic plasticity in the hippocampal area of the brain (for detailed description see the preceding article), (3) behavioural experiments that gave the most direct information about the compounds studied.

With regard to point (2), we have developed an original hypothesis on the hippocampal action of memory-affecting substances (Sugiura *et al.*, 1994; Smriga *et al.*, 1996a). Activity dependent synaptic plasticity in the hippocampus of rodents underlies memory formation and it is generally modelled by using electrical tetanic stimulus as an approximation of learning stimulus. We have used two distinct modes of tetanic stimulus. In intact rats, sub-threshold tetanus evoked a transient, gradually declining form of synaptic potentiation, called short-term potentiation (STP). On the other hand, supra-threshold tetanus evoked stronger, long-lasting potentiation, widely known as long-term potentiation (LTP). The two forms of activity dependent synaptic plasticity share principal characteristics and underly formation of associative memory (Bliss and Collingridge, 1993). In our experimental conditions, LTP represented a saturated phenomenon. Thus, we hypothesized that STP, but not LTP, would be empowered (enhanced) by substances that specifically improve memory formation in intact rats.

Utilizing this framework, we have identified several promising substances for targeting of senile cognitive impairment (Abe *et al.*, 1993; Sugiura *et al.*, 1994; Smriga *et al.*, 1995). Most of the substances were purified from crude natural products used in traditional Chinese medicine.

CHINESE TRADITIONAL MEDICINE AND TREATMENT OF SENILE COGNITIVE DETERIORATION (HOELEN AND OTHER THALLOPHYTIC PLANTS)

Although modern pharmacology has not yet succeeded in addressing senile cognitive impairment, Chinese traditional medicine can display almost 2000 years of thriving clinical practice. Already during the Tang dynasty (618–907 AD), herbal expert Sun Si-Miao wrote a detailed pharmacological syllabus 'Qian Jin Fang' that contained several prescriptions for the improvement of senile cognition. Extracts of thallophytic plants (lichens, fungi, molds, etc.) were especially highly regarded in this respect and the most recommended prescription 'Kai xin san' contained mainly fungus hoelen (*Poria cocos*) (Smriga *et al.*, 1995). 'Kai xin san' was successfully used for the treatment of senile deterioration in medieval Japan and its description is found in the 10th century medical book written by Yasuyroi Tanba.

Chinese traditional medicine often combined several plants to achieve various medical purposes, or to enhance

* Correspondence to: Dr H. Saito, Department of Pharmacology, College of Pharmacy, Nihon University, Chiba 274-8555, Japan.

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Table 1. Summarized effects of thallophytic glucans on formation of hippocampal short-term potentiation (STP) in the dentate gyrus of anaesthetized rats (for details see references)

Glucan	Primary structure	Thallophytic plant	Effect
AS-30 (Smriga *et al.*, 1996c)	Linear α(1–4)	Synthetized glucan (Wako Co., Japan)	–
PC-2 (Smriga *et al.*, 1996c)	Linear α(1–3)(1–4)(3:2)	*Flavoparmelia caperata*	+
PC-3 (Smriga *et al.*, 1996c)	Linear α(1–3)(1–4)(4:5)	*Flavoparmelia caperata*	–
Isolichenan (Smriga *et al.*, 1999)	Linear α(1–3)(1–4)(3:2)	*Cetrarellia islandica*	+
GE-3 (Smriga *et al.*, 1996b)	Linear β(1–6)	*Umbillicaria* spp. *Lasallia* spp.	–
Lentinan (unpublished data)	Branched β(1–3)(1–6)	*Lentinus edodes*	+
Pachyman (Smriga *et al.*, 1996b)	Branched β(1–3)(1–6)	*Poria cocos* (sclerotium)	±
Lichenan (Smriga *et al.*, 1996c)	Linear β(1–3)(1–4	*Cetrarellia islandica*	–

+ Enhancement effect after a single intravenous application.
– No effect.

a single effect without causing side effects. This fact, together with the preventive character of the treatments and poor analytical definition of the plants, caused unjustified disregard for Chinese traditional medicine. Nevertheless, we have continuously attempted to prove that Chinese traditional medicine contains knowledge that could be both experimentally assessable and clinically applicable. Consequently, we attempted to verify experimentally the effects of hoelen in the above-described rodent models of cognition.

The results showed that hoelen had no neurotrophic effects in primary neuronal cell culture. However, chronic oral treatment with a water extract of hoelen (500 mg/kg) improved the learning performance of mice exposed to inescapable stress (Smriga *et al.*, 1996b). In addition, a single oral dose of hoelen (250 mg/kg) significantly enhanced tetanically evoked STP in the hippocampus of rats *in vivo* (Smriga *et al.*, 1995). No effects on LTP and basal synaptic response (response elicited without applying tetanus) were detected.

Secondly, we tried to isolate an active fraction(s) from hoelen. More than 90% of hoelen's content is derived from polysaccharides (pachyman) (Chihara *et al.*, 1970). Although the polysaccharides themselves mimicked the effects of hoelen (data not published), their effects were significantly less pronounced. In spite of that, consistency in the effects of pachyman led to broader screening of thallophytic glucans.

EFFECTS OF THALLOPHYTIC GLUCANS ON HIPPOCAMPAL SHORT- AND LONG-TERM POTENTIATION

Among the numerous thallophytic polysaccharides screened (for summary of polysaccharides and their effects see Table 1), we had identified a new (1–3) (1–4) (3:2) α-glucan, PC-2, isolated from the lichen *Flavoparmelia caperata*. Orally (p.o.) or intravenously injected to rats, PC-2 potently and dose-dependently enhanced tetanically evoked STP in the hippocampus of anaesthetized rats (Smriga *et al.*, 1996c). The efficacy of PC-2 was comparable to the efficacy of crude hoelen, significantly exceeding that of pachyman.

Only STP was enhanced by PC-2. According to the hypothesis mentioned in the first paragraph, since LTP remained unaffected by the glucan, we assumed that, PC-2's effects did not result from pathophysiological increases in neuronal excitability. This conclusion was supported by a lack of PC-2 influences on basal synaptic response.

No effect of PC-2 was observed following its brain infusion, suggesting that the enhancement of STP was peripherally mediated. Several research groups (Pavlides *et al.*, 1993; Smriga *et al.*, 1996a) showed that peripheral stress hormones profoundly modulate memory formation and hippocampal synaptic plasticity. We initially speculated that the effects of PC-2 were dependent on hormonal tonus. In fact, we found that bilateral adrenalectomy suppressed STP in PC-2, but not in vehicle-treated rats (Smriga *et al.*, 1996c).

However, PC-2 is a linear α-glucan, rapidly metabolized into mono- and di-units that may penetrate the blood–brain barrier and enter the brain. Indirect support for this hypothesis comes from a recent study (Matthies *et al.*, 1996) that showed enhancement of synaptic plasticity in the hippocampus by several mono- and di-saccharides. However, another linear α-glucan that is rapidly metabolized into monosaccharides (PC-3) had no effect on STP (Table 1). Moreover, in our experimental conditions, we could not find any significant STP enhancement by mono- and di-units *in vivo*. Thus, at this stage, we do not assume a direct neuronal modulation by PC-2.

The problem is additionally complicated by an unclear relation between the structure of thallophytic glucans and their hippocampal effects (Table 1). Obviously, further studies are needed to give a conclusive answer about a possible structure–effectiveness relationship.

Flavoparmelia caperata is a rare lichen and isolation of PC-2 is an expensive procedure. Thus, from a practical point of view, we have looked for another α-glucan structurally identical to PC-2. Indeed, we have identified isolichenan, a glucan isolated from *Cetrariella islandica*, that differs from PC-2 only in the direction of I_2 reaction (Smriga *et al.*, 1999). *Cetrariella islandica* is widely found in the Northern Europe and the samples used in our experiments were collected in the forests near Helsinki (Finland) in June 1996.

Importantly, similarly to PC-2, peripherally applied isolichenan significantly enhanced STP in the hippocampus of rats *in vivo*, without any effects on basal synaptic transmission and saturated LTP (Smriga *et al.*, 1999).

Taken together, (1) specific drug-induced enhancement of hippocampal STP may serve as an identification method for cognition improving substances, (2) PC-2 and isolichenan potently enhanced STP, without any measurable toxic effects.

EFFECTS OF THALLOPHYTIC GLUCANS ON LEARNING BEHAVIOUR

Behavioural memory assessment belongs to the fundamental paradigms in new drug evaluations. Consequently, we also measured the effects of polysaccharides that positively affected STP in behavioural tests. Due to the relatively easy access to *Cetrariella islandica*, we tested predominantly isolichenan.

Chronically applied (p.o.) isolichenan did not enhance the memory performance of intact rodents in the Morris water maze test and passive avoidance tests (Smriga *et al.*, 1999). Generally, rodents quickly learn cognitive tasks in the Morris water maze, therefore drugs that significantly improve memory performance of intact animals in this test are rare. Hence, to test new cognition-modulating compounds, many experimental groups use memory-impaired animals.

We found that isolichenan significantly improved memory impairment caused by the β-amyloid peptide (fraction 25–35) in the Morris water maze test (Smriga *et al.*, 1999). The Morris water maze is widely recognized as a behavioural counterpart of STP, respectively LTP (McNamara and Skelton, 1993). Thus, our data documented that isolichenan effectively influenced the hippocampally based memory performance not only on the cellular (the enhancement of STP), but also on the behavioural level. Since an increased density of the β-amyloid peptide has been associated with the cognitive impairment found in the Alzheimer's disease (Esler *et al.*, 1999), the efficacy of isolichenan could have future pharmacological applications.

Step through and step down tests are learning tasks utilizing an electric shock as a negative reinforcer. Ethanol pre-treatment caused significant memory impairment in both tests. Isolichenan dose-dependently repaired this deleterious effect of ethanol (Smriga *et al.*, 1999).

Although changes in pain sensitivity and spontaneous movement may secondarily affect learning, neither isolichenan nor ethanol influenced spontaneous movement in tested animals (Smriga *et al.*, 1999). As a result, we assumed that isolichenan intervened in the action of ethanol on learning.

Taken together, the results indicate that isolichenan has preventive effects on memory performance of rodents impaired by β-amyloid peptide and ethanol.

CONCLUSION AND FUTURE PERSPECTIVES

Linear α-glucans (PC-2 and isolichenan), isolated from lichens *Flavoparmelia caperata* and *Cetrariella islandica*, enhanced hippocampal STP in anaesthetized rats. Additionally, isolichenan repaired impaired memory performance in behavioural tasks. It is yet unclear how both glucans affected learning processes. Presently, the most conceivable possibility is a modulation of hormonal input into the hippocampus. Since both the above-mentioned α-glucans could be useful for the development of therapeutic drugs for cognitive impairment, their cellular and molecular targets are currently being investigated.

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