PC-2, Linear Homoglucan with α-linkages, Peripherally Enhances the Hippocampal Long-Term Potentiation

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Purpose. To investigate central effectiveness of PC-2, a glucan from lichen *Parmelia caperata* with α(1–3)(1–4) linkages in the ratio 3:2, with regard to the long-term potentiation (LTP) of evoked potential.
Methods. The extent of LTP, induced by high-frequency stimulation of medial perforant pathway, was evaluated as fractional increase in population spike amplitude in dentate gyrus in anesthetized rats.
Results. Oral and intravenous application of PC-2 resulted in significant enhancement of LTP elicited by a weak, but not by a strong, tetanic stimulation. No influence of PC-2 on basal evoked synaptic potential was found. Bilateral adrenalectomy profoundly suppressed the positive impact of the glucan on the formation of LTP, but showed no effect upon the magnitude and time-course of population spike enhancement in vehicle-treated subjects. Two related α-glucans with different chemical structures did not show any effect comparable to that of PC-2.
Conclusions. Peripheral application of PC-2 significantly enhances LTP in dentate gyrus in rats. Results indicate that the effect of PC-2 might be peripherally mediated and that the specific higher structure of PC-2 is crucial for its biological activity.

KEY WORDS: long-term potentiation; dentate gyrus; PC-2; α-glucan; adrenal steroids.

INTRODUCTION

The hippocampal area, especially dentate gyrus granule cells, is critically important for the initial storage of certain forms of memory. Since long-term potentiation (LTP) possesses several features that make it an attractive cellular model of associative learning (1) and since behavioral studies showed that LTP-like phenomena may be active during real memory formation (2), LTP became the most extensively studied form of central plastic changes.

In traditional oriental medicine, various medicinal products containing glucans were used as remedies against cancer and other diseases (3,4). However, only recently have the biochemical and pharmacological bases of the effects of certain glucans been partly elucidated (4). We showed that a prescription containing hoelen (*Poria cocos* Wolf), which consists predominantly from a β-glucan (4), was not only able to ameliorate the learning and memory deficiency in experimental animals (5), but also qualitatively influence the electrophysiological model of associative cognitive function, i.e. the formation of LTP (6). In the present paper we have focused on purified α-glucan PC-2 (7) (Fig. 1A), present in a wide range of natural lichens (8). Our aim was to find out whether also α-glucans may possess biologically active properties that were presumed for their β-counterparts (4). Due to the above mentioned facts, we have chosen LTP in dentate gyrus of anesthetized rats as a basic model of higher cognitive function. In order to investigate the role of chemical structure, the effect of PC-2 was compared to an α(1–4) glucan and related glucan with different composition of α(1–3)(1–4) linkages (PC-3).

MATERIALS AND METHODS

Materials

PC-2, an α-glucan with (1–3)(1–4) linkages in the ratio 3:2 (number of monosaccharide units = 34–43), and PC-3, a related α-glucan, whose ratio of α(1–3)(1–4) linkages is 4:5 (number of monosaccharide units = 100–130), were isolated from *Parmelia caperata* (8) and the samples were chemically identified by ^{1}H NMR spectra. A synthetized glucan with only α(1–4) linkage with approximate molecular weight of 26500 that served as a reference was available from Wako Pure Chemical Industry, Japan.

Electrophysiological Recordings

Data were obtained from male 7–8 week old Wistar rats (SLC, Japan) that were anesthetized with intraperitoneal (i.p.) injection of urethane (1g/kg) and α-chloralose (25mg/kg) (Sigma). The rats were placed in a conventional stereotaxic device. Installment of both bipolar stainless stimulating electrode (0.25 mm diameter of a single electrode, 0.8 mm tip separation) into the left medial entorhinal cortex and monopolar stainless recording electrode (0.25 mm diameter) into the hilus of the ipsilateral DG was described previously (6). The stimulus was a constant current square-wave pulse, 0.08 ms in duration, applied at intervals of 30 s. Extracellular field potentials were amplified using Nihon Kohden EA-602J amplifiers and displayed on a Hitachi VC-6023 oscilloscope and a pen-printer. When the electrodes were finally placed, stimulus intensity was adjusted to a level that produced population spike of about 50% of the maximal amplitude. After a period of stabilization, drugs dissolved in saline containing 3–5% of dimethylsulfoxide (DMSO), were administered. In the experiments where glucans were administered orally, a volume of 2 ml of the substance was applied via a plastic tube inserted directly to the animal's stomach. In an independent group of experiments, drugs were applied intravenously via a catheter inserted into a femoral vein (injection volume, 200–300 μl). To inject the drug directly to the brain, a stainless steel cylindrical cannula (0.5 mm outside diameter) was placed stereotaxically so that the tip of cannula reached the right lateral ventricle (0.8 mm posterior to bregma, 1.5 mm lateral to midline, 3.7 mm ventral to dura). Intracerebroventricular injection was carried out with a micro syringe connected to the cannula (injection volume, 5 μl; injection time, 2.5 min). Tetanic stimulation was applied at the same stimulus

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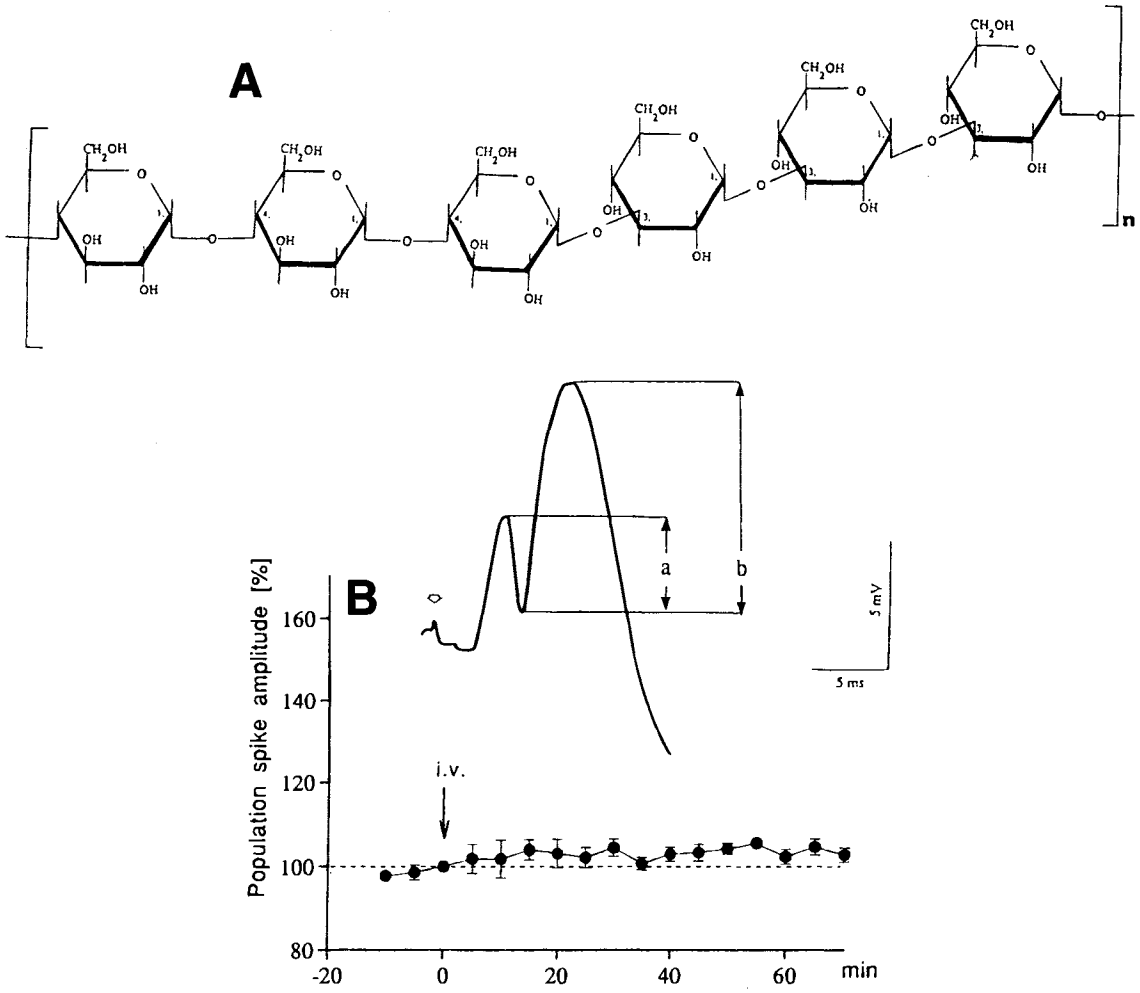

Fig. 1. Effect of PC-2 on the basal evoked potential in the dentate gyrus of intack rats. A: The primary structure of PC-2, showing bonds between adjacent glucose units of the α-glucan skeleton. B: Changes of the basal evoked potential after i.v. injection of PC-2 (1 mg/kg) without application of tetanus. PC-2 was injected at the time indicated by an arrow. Means of 3 rats ± S.E.M. In an inset of the panel B is illustrated a single sweep showing typical evoked potential recorded in the dentate gyrus. Test stimulation was delivered at the time indicated by an arrow. Amplitude of a population spike was defined as an arithmetic average of "a" and "b". Calibration bars are depicted in the right part of the panel.

intensity through the same system of electrodes as used for test stimulation. Two kinds of tetanic stimulations were used in order to produce both LTP (100 pulses at 100 Hz) and decaying form of potentiation (20 pulses at 60 Hz). Within our experimental conditions, the changes in population spike amplitude were profoundly higher and more consistent compared to the changes in the population excitatory postsynaptic potential. All experiments were done at room temperature between 9:00 a.m. and 3:00 p.m.

Surgical Procedures and Corticosterone Assay

In one set of the experiments, rats were anesthetized with sodium pentobarbital (10 mg/100 g, i.p.) (Dai Nippon Company, Japan) and adrenalectomized prior to the electrophysiological procedures. A 1–2 cm midline incision was made on the dorsal hump and using the kidney as a landmark, both adrenal glands were torn away with forceps. Muscle and skin were sutured. Adrenalectomized rats were provided with normal saline as drinking water. In the sham operated rats, all surgical procedures were identical, but adrenal glands were not removed. Electrophysiological procedurs followed exactly 1 week after operation. Blood was collected directly from the heart immediately after electrophysiological procedures. The level of circulating corticosterone was assessed fluorometrically (9) by Hitachi F-4010 fluorescence spectrophotometer. All data was statistically evaluated by one way ANOVA with post hoc Duncan's multiple range test.

RESULTS

As shown in an inset of Fig. 1B, the population spike amplitude was defined as the average of the amplitude from the first positive peak after the rising phase of the evoked potential to the succeeding geometric minimum and the amplitude from the minimum to the second positive peak. PC-2 (i.v., 1mg/kg) did not influence the basal population spike amplitude elicited by a test stimulation (Fig. 1B). A single tetanic stimulation of perforant pathway by high-frequency stimulus of 20 pulses at 60 Hz produced an increase in population spike ampli-

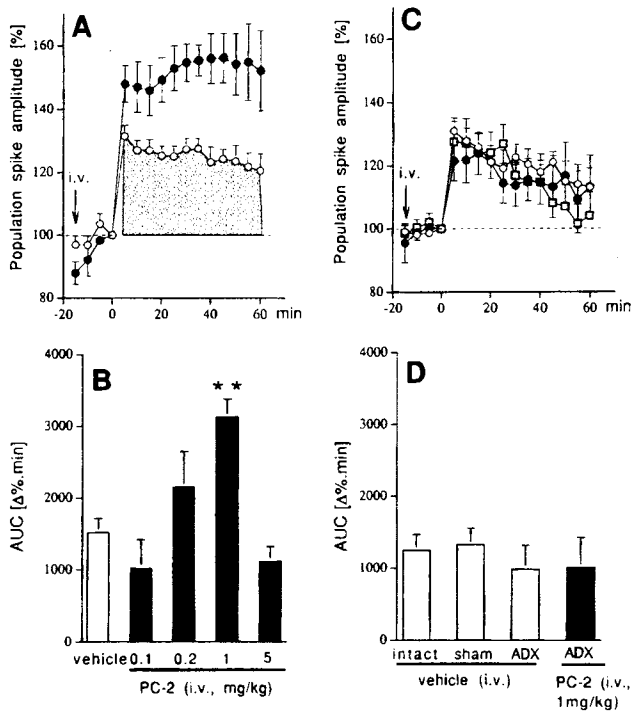

Fig. 2. Effect of intravenous injection of PC-2 on the population spike potentiation induced by a single subthreshold tetanic stimulation (20 pulses/60 Hz) in intact and adrenalectomized (ADX) rats. A: Population spike amplitudes induced by a subthreshold tetanic stimulation in the vehicle (open circle) and PC-2 (closed circle, 1 mg/kg) treated intact rats. Results are expressed as percent differences between basal and tetanically stimulated levels (Δ%). PC-2 was dissolved in 3–5% (v/v) DMSO and applied at the time indicated by an arrow. Tetanic stimulation was delivered at time zero. Means of 3–8 rats ± S.E.M.. The shaded portion below the time-curve corresponds to the AUC from 5 to 60 min after application of tetanus. B: Dose-dependent effect of PC-2 on the population spike amplitude in intact rats after application of single subthreshold tetanus. The AUC from 5 to 60 min after application of tetanus was calculated and defined as an index of LTP magnitude in each group. Means of 3–8 rats ± S.E.M. Asterisks denote statistically significant difference from vehicle injected group. Significance level: $^{**}p < 0.01$ (Duncan's multiple range test). C: Population spike amplitudes induced by subthreshold tetanic stimulation in the vehicle injected intact (open circle), adrenalectomized (open square) and PC-2 injected adrenalectomized (closed circle, 1 mg/kg) rats. Results are expressed as percent differences between basal and tetanically stimulated levels (Δ%). Tetanic stimulation was delivered at time zero. Means of 4–8 rats ± S.E.M. D: The effect of PC-2 on the population spike amplitude in adrenalectomized rats after application of single subthreshold tetanus. The AUC from 5 to 60 min after application of tetanus was calculated and defined as an index of LTP magnitude in each group. Means of 4–8 rats ± S.E.M.

tude in vehicle treated rats (Fig. 2A, open circle). However, potentiation remained above the 120% level for 60 min only in 50% of the total tested animals. Consequently, the above described tetanus was regarded to be subthreshold stimulation for the induction of long-lasting LTP. The shaded portion below the curve was measured as the area under curve (AUC) from 5–60 min after application of tetanus. AUC was defined as an overall index of LTP magnitude. When PC-2 was intravenously applied 15 min prior to the tetanus at a dose of 1 mg/kg (Fig. 2A, closed circle), the studied glucan markedly enhanced post-tetanic potentiation. Potentiation remained above the 120% level during the observation period in all rats tested. Only a weak impact of PC-2 injection was found when the dose was changed to 0.2 mg/kg and no effect was detected at both low (0.1 mg/kg) and high doses (5 mg/kg) (Fig. 2B). Thus, the effect of PC-2 showed a "bell shaped" pattern, with concentration of 1 mg/kg being most effective.

When PC-2 was given intravenously to previously adrenalectomized rats, the enhancement of LTP formation was not observed any more (Fig. 2C, closed circle). Early-decaying potentiation of spike amplitude induced by the subthreshold tetanus in vehicle treated rats was not affected by adrenalectomy (Fig. 2D). The plasma corticosterone levels were assessed immediately after termination of electrophysiological recording. The mean value of 17.2 μg/dl (n = 5) was detected in unoperated rats. Adrenalectomy resulted in a profound decrease in plasma corticosterone (>0.6 μg/dl), in both PC-2 and vehicle treated animals (n = 4–5). Strong tetanic stimulation of 100 pulses at 100 Hz caused persistant potentiation even in vehicle injected rats. Our previous experiments showed that additional changes in the number of pulses, or an increase in frequency of pulses within tetanus did not establish significantly higher potentiation than the one produced by 100 pulses at 100Hz. The described tetanus was used as a convenient source of a stable LTP (10). Intravenous application of PC-2 (1mg/kg) did not affect the fully potentiated population spike induced by this tetanic stimulation (Fig. 3). The effect of an acute oral treatment of PC-2 was also evaluated (Fig. 4). When PC-2 was given at a dose of 250 mg/kg, overall facilitation of population spike amplitude after the application of subthreshold tetanus was significantly enhanced (Fig. 4A), though the effect was somehow weaker than that found after intravenous application. The lower dose of PC-2 (p.o., 125 mg/kg) did not show any detectable influence on population spike amplitude when compared to vehicle treatment (Fig. 4B). To examine the effect of a direct central application, PC-2 was given also intraventricularly. Injection of PC-2 at doses of 10–1000 ng did not affect the magnitude of early-decaying LTP (data not shown).

In order to examine whether the observed biological activity of PC-2 is shared with other glucans, the effects of two related glucans were investigated. Intravenous administration of neither α-glucan PC-3 nor α(1–4) glucan affected population spike potentiation induced by the subthreshold tetanus (Fig. 5).

DISCUSSION

The structure of carbohydrates can be more flexible than the structure of proteins. Overall variability in the unit sugar molecules, differences in the links between them and the presence or absence of branches, support the importance of carbohydrates for the proper function of complex biological interactions (11). The present study for the first time directly proposed central action of a glucan, indicating that peripherally applied α-glucan PC-2 enhances the LTP evoked by subthreshold tetanus in dentate gyrus in vivo. The fully elaborated LTP evoked by suprathreshold tetanus remained unaffected by PC-2, indicating that the treated glucan was without effects upon saturated phenomenon. The character of our recordings did not allow exact distinction between two phenomena produced

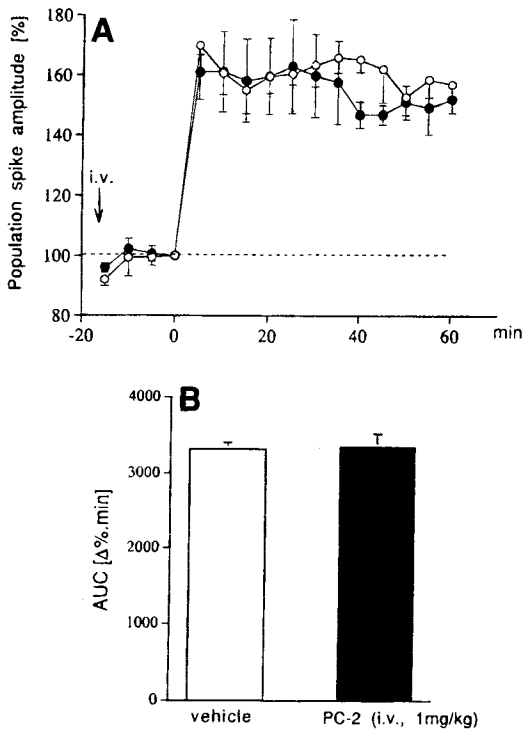

Fig. 3. Effect of intravenous injection of PC-2 on the population spike potentiation induced by a single suprathreshold tetanic stimulation (100 pulses/100 Hz) in intact rats. A: Population spike amplitudes induced by a suprathreshold tetanic stimulation in the vehicle (open circle) and PC-2 (closed circle, 1 mg/kg) treated rats. Results are described as shown in the legend to Fig. 2A. Tetanic stimulation was delivered at time zero. Means of 4 rats ± S.E.M. B: The effect of PC-2 on the population spike amplitude in rats after application of single suprathreshold tetanus. Results are described as shown in the legend to Fig. 2B. Means of 4 rats ± S.E.M.

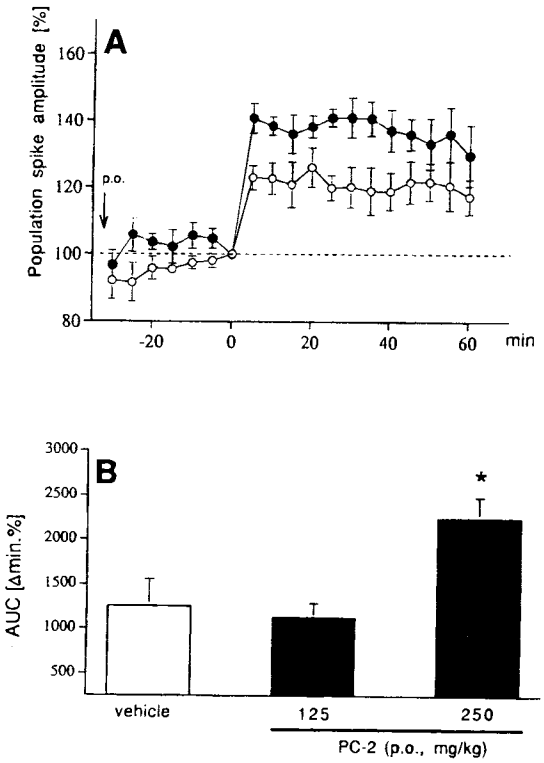

Fig. 4. Effect of oral administration of PC-2 on the population spike potentiation induced by a single subthreshold tetanic stimulation (20 pulses/60 Hz) in intact rats. A: Population spike amplitudes induced by a subthreshold tetanic stimulation in vehicle (open circle) and PC-2 (closed circle, 250 mg/kg) treated rats. Means of 4 rats ± S.E.M. Data are described as shown in the legend to Fig. 2A. B: Dose-dependent effect of PC-2 on the population spike amplitude in intact rats after application of single subthreshold tetanus. Means of 4 rats ± S.E.M. Data are described as shown in the legend to Fig. 2B. Asterisk denotes statistically significant difference from vehicle injected group. Significance level: $*p < 0.05$ (Duncan's multiple range test).

by sub- and suprathreshold tetanus. Decaying form of plastic change may share many properties of LTP, with principal difference being the level of N-methyl-*D*-aspartic acid (NMDA) receptor activity (1). Detected "bell-shaped" concentration pattern corresponds to the same feature found in anticancer action of several polysaccharides (4). As intravenous injection of PC-2 did not influence the synaptic potential evoked by low-frequency test stimulation, glucan was unlikely to affect the information flow between pre- and postsynaptic neurons under normal conditions. Since no effect of intracerebroventricular application of PC-2 was recorded, we assume that the glucan exerted the observed biological activity via modulation of peripheral system(s). This assumption was also supported by the lack of PC-2 effect in adrenalectomized animals. Choosing adrenal glands as the first step in evaluation of a mode of PC-2 action was encouraged by the profound simultaneous impact of adrenal steroids on humoral environment and central cognitive functions (12). This is an important initial assumption with regard to our experiments, since target of PC-2 in an organism (most probably peripherally located) is not known yet. The results from adrenalectomized rats however do not strictly mean that the adrenal axis is a direct target of the studied glucan, since no direct effect of the glucan on plasma corticosterone levels was observed. In spite of the

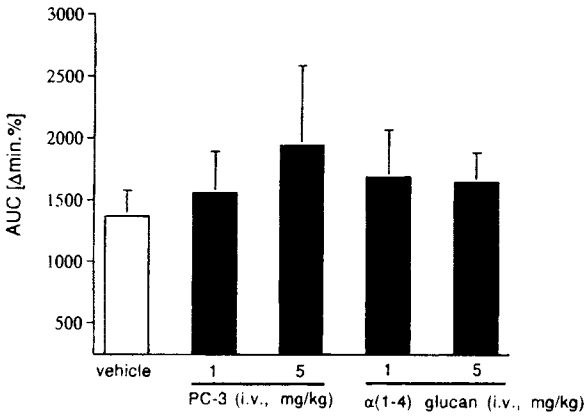

Fig. 5. Effect of intravenous injection of PC-3 and α(1–4) glucan on the population spike potentiation induced by a single subthreshold tetanic stimulation (20 pulses/60 Hz) in intact rats. Dose-dependent effect of PC-3 and α(1–4) glucan on the population spike amplitude after application of a single subthreshold tetanus. Both glucans were dissolved in 3–5% (v/v) DMSO and applied 15 min prior to the tetanus. The AUC from 5 to 60 min after application of tetanus was calculated and defined as an index of LTP magnitude in each group, as illustrated in Fig. 2A. Means of 5–7 rats ± S.E.M.

general skepticism, we were recently able to isolate several active substances from traditional medicines and document their positive impact on neuronal plasticity in experimental animals (10,13). What underlines the qualitative difference of the present paper is basically the peripheral mediation of the PC-2 action. In contrast to PC-2, we found that PC-3 (an α-glucan that differs from PC-2 in terms of the ratio of (1–3)(1–4) linkage and molecular weight) possessed no significant biological activity, at least with regard to hippocampal plasticity. Except the chemical structure (8) we have no information about how peripherally applied glucans are absorbed, metabolized and distributed over tissues. It is difficult to speculate how minor structural differences within two glucans and unequal molecular weight may influence their absorption or distribution. Additionally, no effect of α(1–4) glucan was detected. Hence, the differential effects of the three glucans probably reflect differences in their substantial activities. The importance of the specific higher structure of glucans, similar to proteins, has already been presumed (4).

The mechanisms by which PC-2 enhances LTP formation are unclear yet. Further studies will be aimed at determining both the mode of PC-2 action and physiological meaning of the described observations, which may provide a significant impact upon future biological research of carbohydrates.

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