logical limits[8] when potentiated these may reach dysautonomic magnitudes. An attenuation mechanism is assumed to protect against such changes. We assume that a sympatho-inhibitory mechanism[2,5], co-activated by the LH stimulation together with sympatho-excitatory mechanism is responsible for attenuating the pressor and tachycardic effects, whereas blockage of this mechanism by ATMN results in the potentiation observed. If the sympatho-excitatory mechanism alone is activated, no potentiation is observable.

Descending in parallel from the lateral hypothalamus the excitatory and inhibitory pathways may interact at many levels, the potentiation phenomenon reported here being a sympathetic ganglia manifestation of such interactions. Furthermore, this sympatho-inhibitory mechanism may be identical with the slow muscarinic IPSP described by Libet et al.[9] at the sympathetic ganglia.

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Dopamine synthesis in rat striatum: Mobilization of tyrosine from non-dopaminergic cells

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Summary. Unilateral nigrostriatal lesions in rats that almost totally depleted striatal dopamine had no effect on striatal levels of dopamine's precursor, tyrosine, nor on those of leucine. Since prolonged electrical stimulation of the slices markedly depletes them of tyrosine (1, 2) we conclude that tyrosine can be mobilized from non-dopaminergic striatal cells to augment dopamine release.
Key words. Tyrosine; dopamine, striatum; 6-hydroxydopamine lesions.

The relationship between tyrosine availability and catecholamine synthesis and release has been the subject of recent review[1]. Dopaminergic nerve terminals in electrically-stimulated rat striatal slices rapidly decrease their evoked output of transmitter when the exogenous supply of tyrosine is limiting; concomitantly, tissue tyrosine levels decline markedly whereas levels of other large, neutral amino acids of the same class as tyrosine remain unchanged, and those of dopamine itself fall only slightly[2].

As dopamine neurons comprise only a small proportion of the total cellular mass of the striatum, it therefore seemed unlikely that the tyrosine loss was restricted to these neurons unless their concentrations of the free amino acid were far in excess of those contained in the non-dopamine cells. To explore this question, dopamine nerve terminals were unilaterally destroyed in striata of rats by administration of the neurotoxin 6-hydroxydopamine, and residual tyrosine levels, measured in the lesioned tissue, were compared with levels in the intact contralateral striatum.

Methods. Male Sprague rats (150–200 g) were housed in pairs for at least one week, with food and water supplied ad libitum. Unilateral nigrostriatal lesions were produced by local injection of 6-hydroxydopamine (8 µg/2 µl saline containing 1 mg/ml ascorbic acid) into the anterior substantia nigra of animals maintained under light ether anesthesia. The stereotaxic coordinates used were A-2.4, V-1.6 and L-2.6, according to the atlas of Konig and Klippel[3]. Parglyine (75 mg/kg) was administered i.p. 60 min prior to the injection of 6-hydroxydopamine, in order to prolong the action of the neurotoxin. Animals were decapitated 2 weeks after placement of the lesions, and their striata assayed for dopamine, tyrosine and leucine by methods previously described[2]. Briefly, each demi-striatum was weighed, sonicated in 1 ml of perchloric acid (0.4 M) and centrifuged at 12,000 × g for 20 min. Dopamine was extracted from the supernatant fraction by adsorption to activated alumina, and eluted with acetic acid (0.75 M) using dihydroxybenzylamine (DHBA) as internal standard. Quantitation was by HPLC with electrochemical detection. Dopamine values were corrected for incomplete recovery (80–85%) by comparing the relative peak heights of dopamine and DHBA standards, passed over alumina, with those of the samples. Amino acids were analyzed by the HPLC method of Fernstrom and Fernstrom[4].

Results and discussion. Dopamine, tyrosine and leucine were measured in the left and right striata of rats with right substantia nigra lesions. Data are presented only from animals in which 90% or more of the nigrostriatal fibers were destroyed, as estimated from residual dopamine levels. In lesioned striata, dopamine levels decreased by more than 95% after administration of 6-hydroxydopamine (table). When tyrosine and leucine levels in lesioned and control tissues were compared, no significant differences were observed (table). Levels of leucine were monitored as an additional control because although this amino acid shares certain bio-

Tyrosine and leucine levels in striata of rats with unilateral 6-hydroxydopamine lesions

Amino acid	Lesioned striata (nmol/g)	Contralateral striata (nmol/g)	p
Tyrosine	106.5 ± 19.1	83.9 ± 16.2	NS
Leucine	36.7 ± 16.5	37.4 ± 6.2	NS
Dopamine	1.8 ± 0.5	78.5 ± 4.7	< 0.001

Values expressed are mean ± SEM for 5 animals.

chemical and physical properties with tyrosine, it lacks a role in dopamine synthesis. Since dopaminergic terminals comprise only a small percentage of the cellular mass of the striatum, it can be concluded from these data that the loss of tyrosine from these neurons after electrical stimulation would amount to only a small fraction of the tyrosine present in the slices. Thus, when dopamine neurons are forced (by prolonged electrical stimulation in vitro to release their transmitter, and are not provided with an adequate supply of exogenous tyrosine, additional tyrosine can be mobilized from non-dopaminergic cells as well as from stores within dopamine neurons. It is possible that local glial cells provide one such source of tyrosine, thereby providing nutritional as well as structural support for the dopaminergic terminals, as has been suggested for glia in other systems[5].

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A novel fibrinolytic enzyme (nattokinase) in the vegetable cheese Natto; a typical and popular soybean food in the Japanese diet

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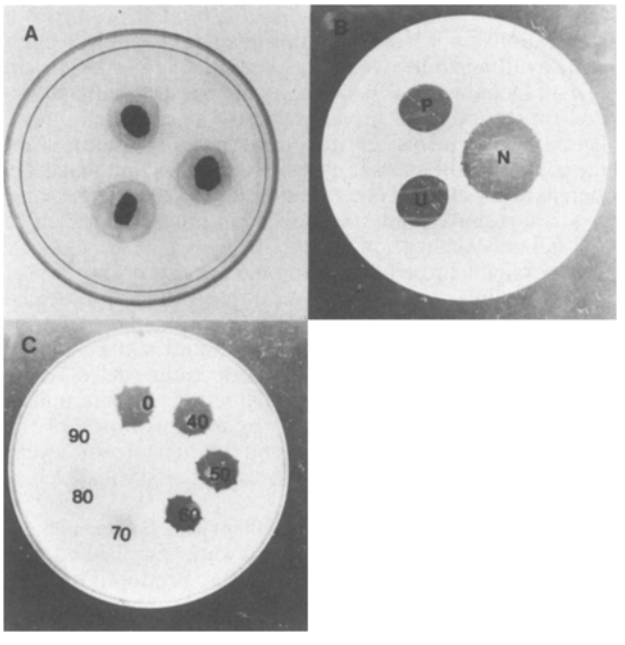
Summary. A strong fibrinolytic activity was demonstrated in the vegetable cheese Natto, which is a typical soybean food eaten in Japan. The average activity was calculated at about 40 CU (plasmin units)/g wet weight. This novel fibrinolytic enzyme, named nattokinase, was easily extracted with saline. The mol. wt and pI were about 20,000 and 8.6, respectively. Nattokinase not only digested fibrin but also the plasmin substrate H-D-Val-Leu-Lys-pNA (S-2251), which was more sensitive to the enzyme than other substrates tried. Diisopropyl fluorophosphate and 2,2,2-trichloro-1-hydroxyethyl-o,o-dimethylphosphate strongly inhibited this fibrinolytic enzyme.
Key words. Natto; soybean food; fibrinolytic enzyme; serine protease.

The vegetable cheese Natto[1] is a typical and popular soybean fermented food in Japan. It has a history extending back more than 2000 years because the special taste and flavor produced by Bacillus natto are well liked[1,2]. Traditionally, it was also used as a folk medicine for heart and vascular diseases, to relieve fatigue and as an anti-beriberi agent[2]. Moreover, a hypotensive effect on SHR rats[3] and prolongation of the life of Ehrlich sarcoma mice[4] have recently been described. However, there is still no detailed report on its component proteases, except for Miyake's preliminary studies with casein and gelatine as enzyme substrates[5,6]. In this report, we demonstrate the presence of a strong fibrinolytic enzyme in Natto for the first time, and describe investigations of some of the enzymatic properties of this novel enzyme.

Materials and methods. The following substances were used: Natto from Samejima Shoji Co. Ltd., Japan; swine pancreas trypsin (type 1) and diisopropyl fluorophosphate (DFP) from Sigma Chemical Co., USA; 2,2,2-trichloro-1-hydroxyethyl-o,o-dimethylphosphate (Neguvon) from Wako Pure Chemical Industries, Ltd., Japan; ε-aminocaproic acid (ε-ACA) and trans-4-aminomethyl-cyclohexanecarboxylic acid (t-AMCHA) from Daiichi Seiyaku Co. Ltd., Japan; human plasmin and urokinase from Green Cross Co., Japan; Bz-DL-Arg-pNA from the Protein Research Foundation, Osaka University, Japan; and pyro-Glu-Gly-Arg-pNA (S-2444), pyro-Glu-Pro-Val-pNA (S-2428), H-D-Val-Leu-Lys-pNA (S-2251), H-D-Phe-Pip-Arg-pNA (S-2238), H-D-Val-Leu-Arg-pNA (S-2266) and H-D-Pro-Phe-Arg-pNA (S-2302) from Kabi Group, Inc., USA. All other chemicals were obtained from commercial sources and were of the best grade available. Amidase activity was estimated colorimetrically using several synthetic amido substrates by an end point method[7]: the reaction mixture (1 ml) consisted of the enzyme sample, 5×10^{-4} M substrate and 0.1 M phosphate buffer containing 0.1 M NaCl, pH 7.4. Fibrinolytic activity was determined by the method of Milstone[8] employing standard fibrin plates[9]. Protein concentration was estimated by the method of Lowry al.[10] using bovine serum albumin (Armour Pharmaceutical Co.) as the reference protein. Gel filtration



Fibrinolytic activity of nattokinase. *A* Natto was applied directly to a fibrin plate. *B* Nattokinase was extracted from 300 g of Natto with 220 ml of saline for 15 min with stirring at 4 °C. The material was filtered through gauze and then centrifuged at 3000 rpm for 10 min. N, nattokinase extract, 21.0 mg protein/ml; P, plasmin standard, 4.0 CU/ml; U, urokinase standard, 100 IU/ml, applied to a fibrin plate, respectively. *C* Nattokinase extract was heat-treated at the temperatures indicated (°C) for 10 min, and then applied to a fibrin plate. Each sample volume was 10 µl, and the incubation time was 18 h at 37°C.