phages with optimal pH 7.0, which was inhibited by chymostatin. However, it is distinct from the present protease because it is not inhibited by leupeptin or antipain and not activated by EDTA or cysteine, and the present protease had no chymotryptic activity under the experimental conditions used.

Literature on the distribution of the neutral thiol protease in macrophages is scarce. Hayashi et al.[17,18] extracted and purified 2 neutral SH-dependent proteases from Arthus skin sites and found them to be derived from perivascular histiocytes and polymorphonuclear leukocytes of rabbits. These hydrolyzed casein optimally at pH 7.1 and hemoglobin at pH 6–7, and had MW of 200,000 and 14,000. These proteases seem to be different from the present protease, because the MW of the latter is 35,000. Next, Cathepsin B, H and L[19,20] are thiol proteases and are widely distributed in mammalian tissues and cells. Cathepsin B is of MW 27,500 with an optimal pH around 6.0, and has peptidyldipeptidase activity. Cathepsin H has a molecular weight of 28,000 with optimal pH 6.8, and has low leupeptin sensitivity. Cathepsin L is of MW 21,000–24,000 with optimal pH 5.5, and hydrolyzed preferentially Z-Phe-Arg-MCA. The present protease has a MW of 35,000 with optimal pH 6.5–7.0 and, hydrolyzed preferentially Boc-Phe-Ser-Arg-MCA more rapidly than Z-Phe-Arg-MCA, and has high leupeptin sensitivity and no peptidyldipeptidase activity under the experimental conditions used. On the basis of MW, optimal pH and some other characters, the present protease would appear to differ from cathepsins B, H, and L. However, in the absence of satisfactorily purified preparations, it is not possible to say anything beyond this description. Further study will clarify the answer to these questions and the biological role of the present enzyme.

The Z-Phe-Arg-MCA cleaving activity in the present macrophage extract has a similar optimal pH and inhibitor profile to cathepsin L except that its MW is 35,000. The exact difference or identity between these two enzymes needs further investigation. The Suc-Gly-Pro-Leu-Gly-Pro-MCA cleaving enzyme has a MW of more than 67,000 with optimal pH 7, and is inhibited by DFP and PCMB and slightly inhibited by leupeptin, chymostatin, antipain, elastatinal and pepstatin, which suggests that the enzyme may be a post-proline cleaving enzyme[21].

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Reduction of high affinity glutamate uptake in rat hippocampus by two polyamine-like toxins isolated from the venom of the predatory wasp *Philanthus triangulum* F.

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Summary. Two components of the venom of the predatory wasp *Philanthus triangulum* F. significantly reduce – to a greater or less extent – the high affinity uptake of glutamate in rat hippocampus. A concentration of 10 μM δ-PTX caused a reduction of 74%, while the other component, β-PTX, at the same concentration, caused a reduction of 18%. Hence the effect of δ-PTX on high affinity glutamate uptake in the hippocampus is comparable with its effect on high affinity glutamate uptake in insect neuromuscular junctions. Contrary to our previous findings that β-PTX has no effect on high affinity glutamate uptake in insect glutamatergic terminal axons, however, β-PTX significantly reduces high affinity glutamate uptake in the hippocampus, albeit less effectively than δ-PTX.

Key words. Glutamate; high affinity uptake; hippocampus; insect toxins; β-PTX; δ-PTX; polyamines.

The β and δ polyamine components of the venom of the wasp *Philanthus triangulum* F. (β-PTX, mol. wt 243 and δ-PTX, mol. wt 435) have been described as regards their effects on glutamatergic insect neuromuscular junctions[1,2]. One of the effects of δ-PTX on these junctions is the inhibition of high affinity glutamate uptake[2]. Glutamate, an excitatory amino acid, seems to be an important transmitter in vertebrate central nervous systems[3–6]. This induced us to investigate the effects of the two toxins on a vertebrate glutamatergic system.

Below a description is presented concerning the results of an investigation of the effects of β and δ-PTX on the high affinity glutamate uptake in the glutamatergic system of the hippocampus.

Material and methods. Hippocampal slices (200 μm) taken from rats (200 g) were prepared and incubated under high affinity uptake conditions[4,7–9]. To establish the effects of β-PTX and δ-PTX on the glutamate uptake in rat hippocampal slices, the latter (6 in 500 μl incubation medium) were preincubated in a

Effects of 10 μM β-PTX and 10 μM δ-PTX on high affinity glutamate uptake in the rat hippocampal stratum radiatum of CA3

	Grain density (g/100 μm²) (SD) (n = 18)	% Inhibition in rat hippocampus	% Inhibition in insect nmj's[c]
Control	14.34 (2.77)		
β-PTX	11.73 (2.45)[a]	18%	0
δ-PTX	3.74 (1.45)[b]	74%	50–85%

[a] Difference from control: p = 0.05 (Student's t-test) [b] Difference from control: p = < 0.005 (Student's t-test) [c] nmj: neuromuscular junction, adapted from Storm-Mathisen[4].

Krebs solution containing either 10 μM β-PTX or 10 μM δ-PTX, for 30 min. Subsequently, during 10 min, the slices were incubated in Krebs solution containing toxin (either 10 μM β-PTX or 10 μM δ-PTX) with the addition of 2.3 μM (50 μCi/ml) ^{3}H glutamate. After incubation the slices were rinsed in Krebs solution (2 × 10 min), fixed in glutaraldehyde (5%), dehydrated and embedded in epon. Control slices were preincubated and incubated as described, not including the addition of the toxin. Preincubation and incubation were performed at 25°C. Autoradiographs were prepared from serial sections (2 μm) through the hippocampal slices, using Kodak Ntb_2 liquid emulsion. After an exposure of 6 days the autoradiographs were developed and stained through the emulsion with toluidine blue.

Results and discussion. The hippocampal formation was used for the investigation of the effects of β-PTX and δ-PTX on the vertebrate central nervous system, since it is a much used and thoroughly investigated cerebral structure with an extensive glutamatergic system[3,4].

It has been shown in the literature that the labeling of hippocampal slices incubated under the conditions described above (2.3 μM (50 μCi/ml) ^{3}H glutamate in Krebs, 10 min) is abolished if sodium ions are omitted from the incubation medium or if aspartate is present in the incubation medium[7,9]. This indicates that high affinity glutamate uptake is responsible for the ^{3}H glutamate accumulated in the hippocampus and that no receptor-bound ^{3}H glutamate contributes to the labeling. Moreover, incubation conditions for the demonstration of receptor-bound ^{3}H glutamate are completely different[10]. The possibility of a low affinity uptake system contributing to the accumulated ^{3}H glutamate may be neglected, considering the low concentration of ^{3}H glutamate in the medium (2.3 μM, 50 μCi/ml) and the short incubation time (10 min). Therefore this established and acknowledged method was adopted for the present investigation of the effects of the two polyamine insect venom compounds on high affinity glutamate uptake.

To exclude erroneous results owing to slow penetration of either ^{3}H glutamate or the toxins, grain counts were made above the stratum radiatum of the CA_3 region from serial sections through both control and toxin treated hippocampal slices (200 μm). The aforementioned counts revealed that the grain counts from serial sections through one hippocampal slice were identical. This holds for control slices as well as for toxin-treated slices. The results of this investigation were interpreted as an indication that the penetration of both glutamate and the two toxins into the slices was sufficient and that neither the amount of glutamate nor the amount of either of the two toxins were limiting factors. The grain counts in the 2-μm sections above the stratum radiatum of the CA3 region revealed that both β-PTX and δ-PTX significantly reduce the amount of accumulated glutamate (table). The greatest reduction of high affinity glutamate uptake (74%) was observed in slices treated with δ-PTX. A small, but still significant reduction (18%) was observed in slices treated with β-PTX. The 74% reduction of glutamate owing to the action of 10 μM δ-PTX is comparable to the inhibition observed in the glia and terminal axons of insect neuromuscular junctions[2]. However, in insect neuromuscular junctions no effect of β-PTX can be observed, whereas in hippocampal slices β-PTX reduces the glutamate uptake significantly (18%).

The above observations indicate that the polyamine-like δ-PTX and possibly β-PTX, both of which affect the glutamatergic transmission in insects[1], are also active in the glutamatergic transmission in rat brain. The action of these toxins with their polyamine structure is in agreement with the observation that polyamines act as specific uptake inhibitors in synaptosome preparations[11].

Hence these toxins and their structural analogues may serve as pharmacological tools for the investigation of glutamatergic transmission processes, as it was possible to use natural toxins for the elucidation of cholinergic transmission processes[12].

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The role of the autonomic nervous system in mediating pancreatic endocrine responses to arginine in the calf[1]

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Summary. The release of insulin which occurred in response to arginine, in the conscious calf, differed from that which occurs in response to glucose in that it was not significantly affected by either adrenergic or muscarinic blocking agents. Release of pancreatic glucagon was reduced by pretreatment with phentolamine.
Key words. Arginine; endocrine pancreas; autonomic nervous system.

The release of insulin from the pancreas which normally occurs in response to hyperglycemia in the conscious calf is mediated largely via the parasympathetic innervation[2]. In addition, it is effectively abolished by pretreatment with propranolol alone (0.25 mg/kg i.v. 10 min before the glucose challenge) but not by combined pretreatment with both propranolol and phentol-