of N-methylglucamine solution in 0.9% NaCl with 4% PVP. Each group consisted of 20 mice. The death rate after the poisoning was measured. All animals were observed over a period of 7 or 14 days. The results were calculated over a program on the Olivetti P 652 with the 'Vierfelder χ^2 test', corrected by Yates. If the expected values were 5, then the exact p-values were calculated by Fisher's test.

Results. The toxic doses of α-amanitine and phalloidine were chosen so that 75–100% of the controls will die. The treatment with silybin 1 h before the intoxication reduced the death rate in both experiments significantly. Under the same conditions taxifolin, coniferyl alcohol, fisetin and (+)-catechin were ineffective[8].

Discussion. The toxins used from the death-cup toadstool (Amanita phalloides) show different modes of action. The site of action of phalloidine is the plasma mebrane of the liver cell[9]. Death in small rodents follows the i.p. application of a lethal dose within 2 or 3 h. The liver is swollen to its maximum, light-microscopically, one is impressed by massive vacuolization of the liver cells, and some of the vacuoles contain erythrocytes[10].

α-Amanitine blocks the RNA-polymerase B in the eukaryotic cells[11]. The absence of the synthesis of ribonucleic acids can explain the long time-effect sequence between poisoning and the manifestation of the damage in the parenchyma. Mice die not before 2–4 days after the intoxication. From experiments carried out with phalloidine and silybin, on the isolated perfused liver or on isolated hepatocytes, it has been shown that silybin protects the liver cell by interacting with the outer cell membrane[6,12]. The prophylactic and therapeutic use of silybin against the toxic action of phalloidine and α-amanitine underlie, in vivo, the same temporal conditions. Therefore, it may be assumed that silybin hinders α-amanitine from penetrating into the cell.

The identification of the molecular structure of silybin a few years ago[1] has shown that this compound cannot be classified into any of the known groups of natural compounds, but rather is a new and peculiar chemical structure. As the presented experimental results show, silybin occupies a special position, also in a pharmacological view.

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Current(I)-voltage(V) relationships of the neuromembrane of an identifiable giant neurone of an African giant snail (*Achatina fulica* Férussac) in the presence of an inhibitory tripeptide, L-Lys-L-Phe-L-Tyr

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Summary. An inhibitory tripeptide, L-Lys-L-Phe-L-Tyr, caused membrane hyperpolarization of the TAN (tonically autoactive neurone) resulting in an elevated firing level. The tripeptide, however, did not markedly affect either the TAN I-V curve or the firing pattern obtained by transmembrane triangular current injection.

In a previous paper[2], we reported that a tripeptide, L-Lys-L-Phe-L-Tyr, obtained as a fragment of physalaemin (a hypotensive endecapeptide[3,4]) had an inhibitory effect on the excitability of a giant neurone (the TAN, tonically autoactive neurone) identified in suboesophageal ganglia of the African giant snail, *Achatina fulica* Férussac. A dipeptide, L-Phe-L-Tyr, also had the same effect on the same neurone[5], although the inhibition was less than that obtained with L-Lys-L-Phe-L-Tyr. None of the amino acids involved, however, had any effect on the TAN excitability when tested individually[6].

In the present study, we used a transmembrane triangular current injection method to measure current(I)-voltage(V) relationships (I-V curve) of the TAN neuromembrane. This system was then used to study the effect of the application of L-Lys-L-Phe-L-Tyr. We studied also the effect of this tripeptide on both the TAN firing level and the firing pattern produced by a depolarizing current injection.

Material and methods. The pharmacological characteristics and the localization of the neurone examined (the TAN) in the suboesophageal ganglia have been described in previous papers[7-9]. The electrophysiological methods adopted in the present study have also been described in detail in these papers. 2 glass microelectrodes were implanted in the TAN soma: one to record the intracellular potential and one to inject a current into the soma. The I-V curve, the firing level and the firing pattern of the neurone were measured simultaneously by the injection of a transmembrane triangular current (hyperpolarizing, depolarizing and hyperpolarizing, 120 sec/cycle)[10]. L-Lys-L-Phe-L-Tyr (donated by Dr A. Inoue of Daiichi Pharmaceutical Co., Tokyo) was dissolved in the snail's physiological solution[11] and applied directly to the dissected ganglia (the bath application).

Results and discussion. Figure 1, A and B, show I-V curves of the TAN neuromembrane obtained under the following conditions: A, in the physiological state; B, 3 min after the application of L-Lys-L-Phe-L-Tyr in a concentration of 2×10^{-4} kg/l. There was no distortion of the current intensity recording, indicating that electrical rectification was not a problem with the current injecting microelectrode used. In the physiological state (A), spontaneous spike

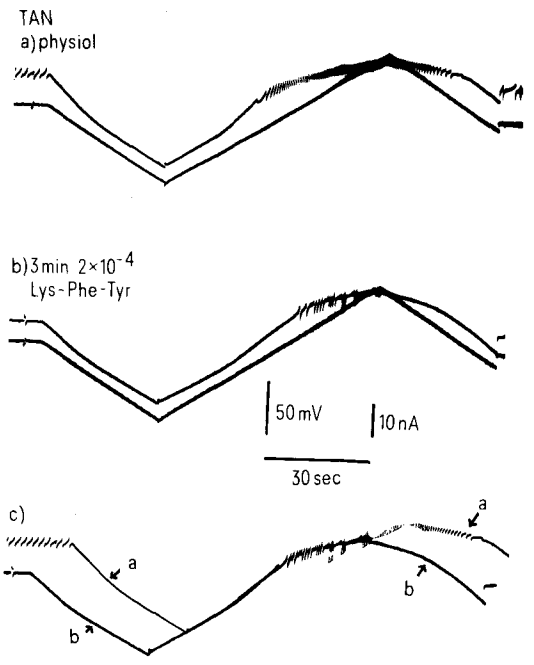

Fig. 1. Changes of the current(I)-voltage(V) relationships (I-V curve) of the TAN (tonically autoactive neurone) membrane caused by L-Lys-L-Phe-L-Tyr. A triangular current (hyperpolarizing, depolarizing and hyperpolarizing, 120 sec/cycle) was applied into the TAN soma to obtain the I-V curve. A: in physiological state. B: 3 min after the application of L-Lys-L-Phe-L-Tyr at 2×10^{-4} kg/l. The upper traces of A and B show the TAN potential shift produced by a triangular current injection. The lower traces of A and B show the intensity of the applied current. Left vertical bar: calibration of the potential shift (50 mV). Right vertical bar: calibration of the applied current intensity (10 nA). Horizontal bar: time course (30 sec). In C, the 2 I-V curves, A and B, have been superimposed by using the firing level as the reference point.

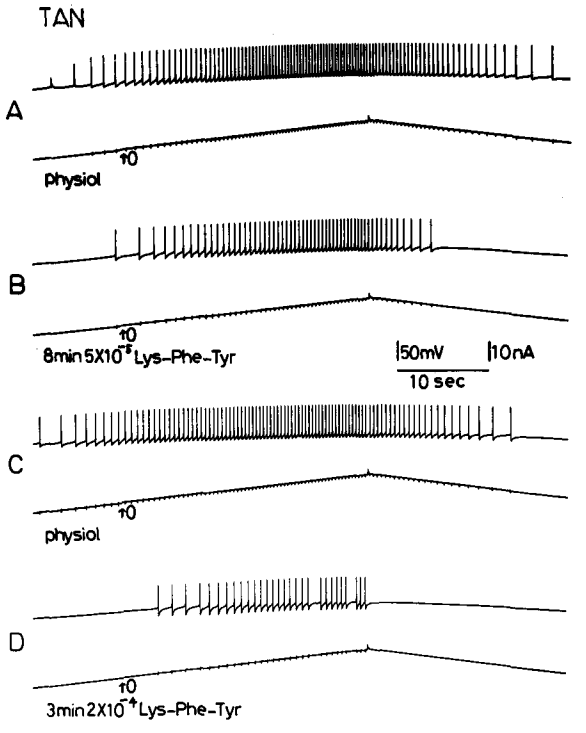

Fig. 2. Changes of the TAN firing level and the firing pattern obtained by a depolarizing current injection in the presence of L-Lys-L-Phe-L-Tyr. The upper traces of A, B, C and D show the TAN potential. The lower traces of A, B, C and D show the intensity of the applied current. The 'arrow 0' marked on the lower traces indicates the 'zero' intensity of the applied current. A: in the physiological state. B: 8 min after the application of L-Lys-L-Phe-L-Tyr at 5×10^{-5} kg/l. C: in the physiological state. D: 3 min after L-Lys-L-Phe-L-Tyr at 2×10^{-4} kg/l. Left vertical bar: calibration of the potential (50 mV). Right vertical bar: calibration of the applied current intensity (10 nA). Horizontal bar: time course (10 sec).

discharges were present before the hyperpolarizing current was applied. The presence of L-Lys-L-Phe-L-Tyr at 2×10^{-4} kg/l, however, resulted in a neurone that was silent even before current-induced hyperpolarization. In C of this figure, the 2 I-V curves (A and B) have been superimposed using the firing level as the reference point. The 2 I-V curves are seen to be concordant over a large proportion of the neuronal polarization. Therefore, we conclude that the TAN I-V curves were not markedly modified by the application of L-Lys-L-Phe-L-Tyr at 2×10^{-4} kg/l, a concentration causing hyperpolarization and cessation of the spontaneous spike discharges of the TAN potential.

Figure 2 shows the TAN firing pattern induced by the depolarizing triangular transmembrane current injection in the physiological state (A and C) and in the presence of L-Lys-L-Phe-L-Tyr (B and D). The 'arrow 0' marked on the applied current intensity recording indicates a current intensity of 'zero'. In the presence of L-Lys-L-Phe-L-Tyr, the firing level of the neurone was clearly elevated compared to that of the physiological state. This was because of membrane hyperpolarization. The firing pattern of the neurone, however, was not markedly affected by the application of the tripeptide. Occasional small disorders of the firing pattern were observed in the presence of a high concentration of the tripeptide, as shown in figure 2, D. It is concluded, therefore, that L-Lys-L-Phe-L-Tyr caused hyperpolarization of the TAN membrane, but did not affect either the production of the action potential or the firing pattern induced by the depolarizing triangular current injection.

The tripeptide in a concentration sufficient to cause cessation of the TAN spontaneous spike discharges did not result in any modification of the TAN I-V curve. The localization on the TAN neuromembrane of receptors for the tripeptide is possibly so remote from the recording point of the intracellular potential that the modification of the membrane resistance caused by this substance could not be detected in the method employed.

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