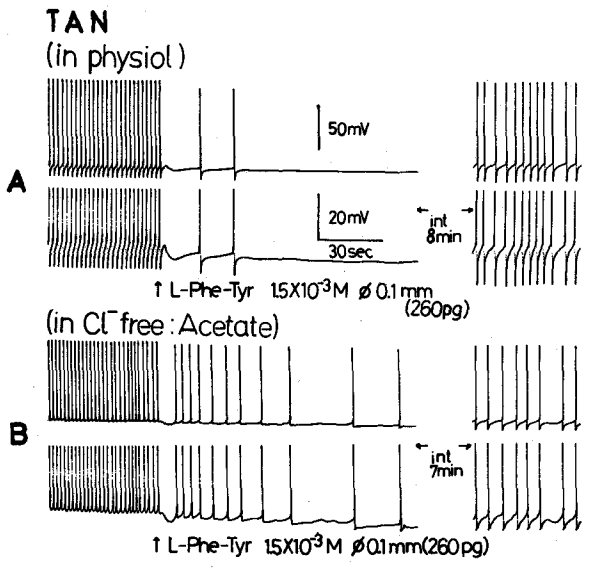

Fig. 2. Effect of L-Phe-L-Tyr on the TAN biopotential in both physiological state (A) and chloride-free medium (B) (microdrop application). The upper traces of A and B are full-spike recordings of the PON biopotential by a pen-writing galvanometer. The lower traces of A and B are high amplification recordings of the upper traces (spike peaks have been cut by an electronic voltage clipper). In both physiological state (A) and chloride-free medium (after the perfusion of the isotonic acetate [chloride free] solution for at least 15 min) (B), a microdrop (100 µm in diameter) of 1.5×10^{-3} M (5×10^{-4} kg/l) L-Phe-L-Tyr solution (the total amount of the dipeptide estimated to be about 260 pg) was applied on the TAN surface (arrow). Upper vertical bar, calibration for the upper traces (50 mV). Lower vertical bar, calibration for lower traces (20 mV). Horizontal bar, the time course (30 sec).

TAN inhibition caused by the dipeptide is not dependent on the permeability increase of the neuromembrane to chloride ions, since the presence of chloride ions in the medium is not necessary for the dipeptide to show the inhibitory effect.

It was previously reported[9] that each amino acid, of which the inhibitory peptides mentioned consist, had no effect on the TAN. Of the substances examined in the present study, therefore, L-Phe-L-Tyr is thought to be the essential structure for producing the effect. Although we can cite the pioneering works of Kakimoto et al.[10,11] on the presence of glutamyl dipeptides in the mammalian brain, yet, in general, the presence and the role of oligopeptides in the nervous tissue remains obscure. We propose the possibility that the inhibitory effect of L-Phe-L-Tyr on the TAN might be physiological, the dipeptide acting perhaps as a neurotransmitter or as a structurally analogous substance to an unknown transmitter. Further studies will have to be performed in order to clarify this area of research.

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Inhibitory effect of taurine on decrease in the inotropic action of ouabain at high concentrations in isolated atria

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Summary. In vitro, taurine was shown to inhibit the decrease in the inotropic effect of ouabain at large doses in the normal and also low K^+ medium in which this decrease in the inotropism of ouabain was facilitated. This inhibitory effect of taurine was, at least in part, due to the inhibition of the efflux of intracellular K^+ in the isolated heart.

A large quantity of taurine was found in mammalian heart[1–3], and yet little is known about its function in the heart[4]. It has been indicated that taurine acts as an antiarrhythmic in vagotomized dogs[5], rats[6] and guinea-pigs[7]. The antiarrhythmic effect was suggested to be, at least in part, due to prevention by taurine of the efflux of intracellular K^+ associated with some drug-induced arrhythmias[6,8]. Since taurine failed to influence Na^+, K^+-ATPase activity or an interaction between ouabain and Na^+, K^+-ATPase[6,9], this enzyme seemed not to be concerned in the antiarrhythmic action of taurine.

In isolated hearts, however, taurine, being positively inotropic in guinea-pig auricles in both normal and low Ca^{++} medium[10], potentiated positive inotropic effects of strophanthin-K[11] and ouabain[12], and inhibited a decrease of contractile force by Ca^{++}-free media[13] in these preparations. It was indicated that the potentiating effect of taurine on the positive inotropic effect of ouabain was, to some extent, related to an accumulation of intracellular Ca^{++} in the taurine-loaded heart[12].

Thus, the interest in these reports comes from the findings that taurine antagonized the cardiotoxic action of digitalis, but potentiated the positive inotropic action. In addition, any antagonisms between taurine and cardiac glycosides on isolated heart preparations have never been reported. The present author, therefore, attempted to demonstrate an inhibitory action of taurine on a decrease of the inotropic action in high concentrations of ouabain in isolated atria. Since reduction of serum K^+ levels increased the positive inotropic response to ouabain[14] and enhanced digitalis toxicity[15] and cardiac uptake of ouabain[14], it was also examined whether or not the inhibitory effect was influenced in a low K^+ medium.

Methods and materials. Detailed methods have been described elsewhere[7]. Briefly, left atrial strips, prepared from male guinea-pigs (250–350 g) were driven by electric

stimulation in Tyrode solution. Changes by ouabain in the contraction amplitude were recorded isotonically for 15 min or more, and thereafter the preparation was subjected to determination of intracellular K^+ contents, on an atomic absorption spectrophotometer (Perkin-Elmer Model 303). In some experiments, taurine was added to an organ bath at final concentrations of 0.5 and 3.0 mM, 10 min prior to ouabain. Isotonicity in the low K^+ (2.0 mM)-medium was adjusted by NaCl. Total and extracellular water was determined by the antipyrine method[16] and the inulin method[17], respectively. Drugs used were taurine (Taisho Pharmaceutical Co., Ltd, Tokyo) and ouabain (E. Merck, Darmstadt, Federal Republic of Germany).

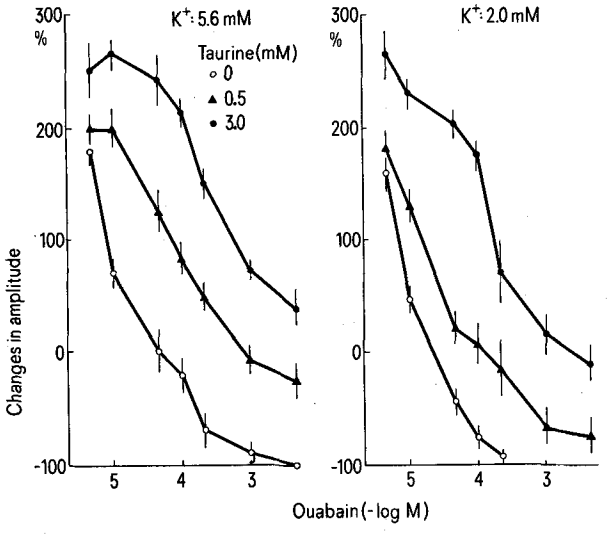

Fig. 1. Effect of ouabain on the contraction amplitude in the normal (left) and the low K^+ (right) media in the absence (○) or the presence of taurine at doses of 0.5 (▲) and 3.0 mM (●). Ordinate, percent change in the amplitude, abscissa, molar concentration of ouabain (-log scale). Bars represented SEM in 15–20 instances.

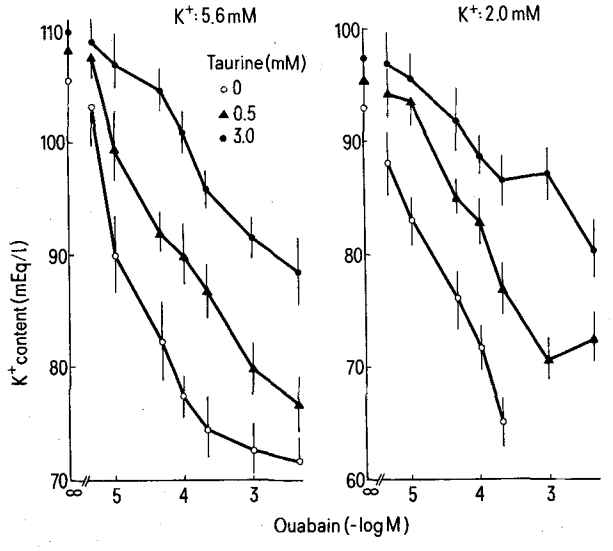

Fig. 2. Effect of ouabain on the intracellular K^+ concentration. Ordinate, K^+ content expressed in mEq/l of intracellular water. Same legends as in figure 1.

Results and discussion. When ouabain was added to the normal medium at final concentrations ranging from 5 μM to 5 mM, the contraction amplitude of the heart increased without visible evidence of contracture. A maximal inotropic effect was obtained 3–8 min after ouabain. After the maximal inotropism, contractility became smaller and finally nondetectable, and this later stage, which was referred as to a toxic stage of ouabain in the following discussion, was often accompanied by development of contracture and increase in automaticity.

Figure 1 shows percent of changes on the contraction amplitude 15 min after ouabain (an pre-ouabain level = 0%). The decrease in the inotropic effect of ouabain at doses more than 0.05 mM was facilitated by lowering the K^+ contents from 5.6 mM to 2.0 mM in the incubation medium, but inhibited by taurine at the doses of 0.5 and 3.0 mM which was added to the normal and low K^+ medium, 10 min prior to ouabain. This inhibitory effect of taurine was dose-dependent within the doses used. The development of contracture and automaticity were also depressed by taurine. Since taurine was suggested to act as the antiarrhythmic by preventing the efflux of intracellular K^+[6,8], the intracellular K^+ concentration was determined after the isolated preparation was incubated with ouabain for 15 min. Ouabain reduced the content of intracellular K^+ (figure 2) in the normal and low K^+ media in which the decrease of the K^+ contents by ouabain was greater than that in the normal. The reduction by ouabain of intracellular K^+ was inhibited by taurine in both media, and the inhibition by taurine of the toxic stage (the decrease of the inotropic action) in the high concentrations of ouabain paralleled the inhibition of the efflux of intracellular K^+. Although the data available at the present work do not allow an unequivocal answer, it could be suggested that the inhibiting effect of taurine on the decrease of the ouabain-induced inotropism was, at least in part, due to the inhibition of the efflux of intracellular K^+. In this connection, Huxtable[18] suggested that taurine was of value in controlling arrhythmias by depressing hyperirritability caused by loss of intracellular K^+, and that any theory involving K^+ retention by taurine might propose a mechanism whereby the re-uptake of K^+ by the heart was increased, by a route other than

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the Na^+, K^+-pump, rather than a decreased K^+-conductance out of the atrial cell. However, the possibility should not be excluded that a stimulating effect of taurine on the Na^+, K^+-pump was a plausible explanation for the finding reported.

Taurine alone, at the doses used, did not affect contractility of the heart and amounts of intracellular K^+ in both media. Since it has been shown that, at the stage at which development of contracture was induced by large doses of ouabain, there were an increase in Ca^{++} contents and a decrease in K^+ contents in hearts[19], the possibility should also not be excluded that taurine might prevent the increase of the intracellular Ca^{++} content at the toxic stage (the decrease in the inotropism after the large doses of ouabain). On the other hand, the present authors[12] reported that a combined treatment of taurine at the dose of 3.0 mM, and ouabain at doses ranging from 0.5 μM to 2 μM under the same experimental conditions as the present, resulted in both potentiation of the positive inotropic effect and an increase in intracellular Ca^{++} contents. Other authors have also reported that taurine promoted a myocardial uptake of Ca^{++}[13] and slowed a rate of myocardial loss of Ca^{++}[20]. Consequently, the inhibitory effect of taurine on the decrease of the inotropic action of ouabain at the doses used might not be concerned in movement of intracellular Ca^{++}.

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Effect of harmaline on the cerebello-rubral system

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Summary. Harmaline induces synchronous rhythms in both the cerebellum and the red nucleus of the rabbit. The level of synchronization is lower in the red nucleus than in the cerebellar cortex, probably because the cerebello-rubral pathway and the red nucleus neurons only participate poorly in the harmaline-induced olivo-cerebellar rhythm.

Previous investigations[2–7] have shown that under the influence of central depressants (barbiturates in particular), strychnine and tetanus intoxication, synchronous frequencies can always be recorded from the cerebellum (CB) and red nucleus (NR), whereby NR-neurons fire in bursts that are correlated with the respective cerebello-rubral rhythm. Evidence has been presented that the synchronous firing of these neurons and the NR-rhythm are initiated by cerebello-rubral pathway[2,5]. The discharge pattern of NR-cells is so distinctly correlated with the cerebellar rhythm, as if these units were under the synaptic drive of the cerebellar pathway only.

In studies on tremor mechanisms, Lamarre et al.[8,9] have proved that the harmaline-induced 6–12 Hz tremor is generated by rhythmic firing of olivary neurons; these impulses are transmitted to spinal levels mainly by the

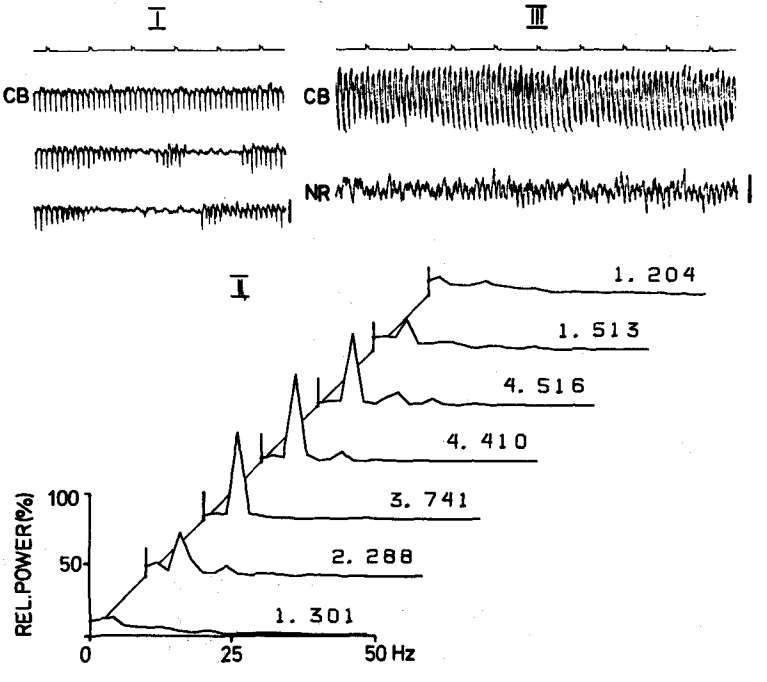

Fig. 1. Effect of harmaline on the electrical activities of the cerebellum (CB) and red nucleus (NR). *I* Continuous record (EEG) from the CB; calibrations: 0.2 mV and 1 sec. *II* Power spectra of the electrocerebellogram. Each spectrum represents the analysis of a 10-sec recording sample with a frequency resolution of 2 Hz. Numbers: total power (μV^2) in arbitrary units. *III* Simultaneous records from the CB and NR; calibrations: 0.2 mV and 1 sec.