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EFFECTS OF UROPHYSIAL EXTRACTS ON THE CARDIOVASCULAR SYSTEM OF THE CARP, Cyprinus carpio

(caudal neurosecretory system/cardiovascular system/carp)

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Urophysial extracts (UE) of carp induced (1) dose-dependent vasopressor effect on the carp, Cyprinus carpio, (2) dose-dependent contraction of the helically cut strip of carp ventral aorta, which contraction was not mediated by the adrenergic or cholinergic system, and (3) a positive inotropic effect on the arterial strip of the carp. These results suggest that increase in blood pressure elicited by UE is at least partially mediated through the effects of UE on the contraction of the ventral aorta, probably on the contraction of the arterioles, and on the enhancement of the contractile force of beating on the atrium. Dose dependent contraction of the aortic strip was elicited by partially purified urotensins I (UI) and II (UII) as well as UE of the carp. The contraction of the aortic strip by UE was almost entirely due to UII contained in the UE.

Urophysial extracts (UE), i.e. acid extracts of the urophysis which is a neuroheamal organ of the caudal neurosecretory system of bony fish, show various biological activities (1, 2). One of the activities is a vasopressor effect in the eel (3-6); the experiments were carried out in the eel using UE of another species. There have been no experiments on the vasopressor activity of UE in any other species.

In addition to vasopressor activity, UE induces contraction in various smooth muscles of the fish Salmo gairdnerii, Lebistes

reticulatus, and Gillichthys mirabilis (7, 8). It is possible, therefore, that the vasopressor activity of UE is mediated by the contraction of the smooth muscles of the vascular system. To clarify the mechanism of vasopressor effects, effects of UE on the atrium should also be studied. Preliminary results on contractile activity of isolated peptides from Catostomus commersoni UE in ventral aorta of Catostomus c. were reported previously at the 9th International Symposium on Comparative Endocrinology (9).

The purpose of the present study is (a) to investigate the vasopressor activity of carp UE in the carp and (b) to analyze the mechanism involved in the vasopressor activity of UE, using isolated preparations of carp ventral aorta and atrium. Preliminary results of these experiments have been reported previously (10).

MATERIAL AND METHODS

Carp, Cyprinus carpio, of both sexes were obtained from a commercial source. They were about 40 cm in total length and about 900 g in body weight.

Preparation of UE

Urophyses of the carp were cut out and dehydrated in acetone. The aceton-dried urophyses were homogenized in 0.25% acetic acid, heated in a bath of boiling water for 3 min. and centrifuged at 3,000 rpm for 10 min. (11). The supernatant, diluted to a concentration of 1 mg of acetone-dried tissue per 1 ml, was used as UE. The UE was stored in a freezer at -20°C. UI and UII fractions from carp urophyses, prepared by chromatography through Sephadex G-25 eluted with 0.25% acetic acid were kindly donated by Dr. T. Ichikawa, Tokyo Metoropolitan Institute of Neurology. UI activity of the UI fraction was 500 mU/ml and UII activity of the UI fraction was lower than 17 mU/ml. UII activity of the UII fraction was 150 mU/ml and UI activity was lower than 50 mU/ml. One unit of activity is the activity induced by 1 mg of acetone-dried carp urophysis in rat antidiuretic assay for UI (12) and in trout rectum assay for UII (13).

Measurement of blood pressure

The ventral aortic blood pressure, as well as the heart

rate, of the conscious carp (n= 5) were measured in the bulbus arteriosus. After anesthesia with tricaine (MS 222, Sandoz) (1/3,000), heparin (500-800 i.u./kg BW) was injected into the caudal vein through a silastic tubing (0.012 in. ID) and a needle connected with a polyethylene tubing (PE 50) was inserted into the bulbus arteriosus. The free end of the polyethylene tubing was connected to a transducer (MP-4, NIHON KODEN), which was connected to a polygraph (RM-150, NIHON KODEN). All animals were allowed at least 6 hours rest before commencement of recording. UE was injected intravenously through the caudal vein.

Experiments using the ventral aortic strip

The ventral aorta was cut free of surrounding tissue in a paraffin-based Petri dish, and spiral strips (about 1.5 cm in length and 2 mm in width) of aorta were prepared. Each strip was mounted vertically in a 5-ml organ bath containing Cortland's solution (14). The composition of the Cortland's solution was as follows (mM): NaCl 124, KCl 5, $CaCl_2$ 1.5, $NaHCO_3$ 12, NaH_2PO_4 3 and glucose 5.

The solution was continuously aerated with 95% O_2- 5% CO_2 and the temperature was maintained at 15°C. This temperature was found to be optimum for spontaneous beating of isolated atrial preparations in a preliminary experiment. The upper end of the strip was connected to a force displacement transducer (SB-1T, NIHON KODEN) with a thread and the change in isometric tension was recorded. The initial tension of 0.5 g was loaded. An equilibration period of at least 90-min. was allowed for each preparation before the start of the experiment.

Observations were made on 4 strips by adding the samples cumulatively into the bath. The strip was allowed to rest for at least 40 min. before the next addition of samples.

To compare the maximum contraction of the strip, UE (10^{-9}-10^{-5} g/ml, final concentration) and acetylcholine chloride (ACh) (10^{-9}-10^{-4} M, final concentration) were used. Four strips were used for this experiment.

To examine whether the contraction of UE is mediated by the autonomic nervous system or not, atropine sulfate (10^{-9} and 10^{-8} M, final concentration) or phentolamine mesylate (10^{-6} M, final concentration) was added to the bath 15 min prior to the addition of UE.

Experiments using the atrial strip

Seven atria were cut free of surrounding tissues in a paraffin-based Petri dish. Each atrium was cut through the midline and a strip without a pacemaker area, located between the entrance of the venous sinus and the atrio-ventricular ostium (15), was prepared. The preparation was mounted and the contractile force was recorded in a similar manner to the ventral aortic preparation. The initial tension on the atrial strip was 0.3 g. The strip was stimulated with rectangular pulses of 3 msec duration at 0.1 Hz generated by an electrical stimulator (SEN-3201, NIHON KODEN). The voltage of the pulse was 3 times higher than that of the threshold. An equilibration period of at least 120-min. was allowed for each atrial preparation before the start of the experiment. The effect of UE was analyzed using a paired-sample randamization test.

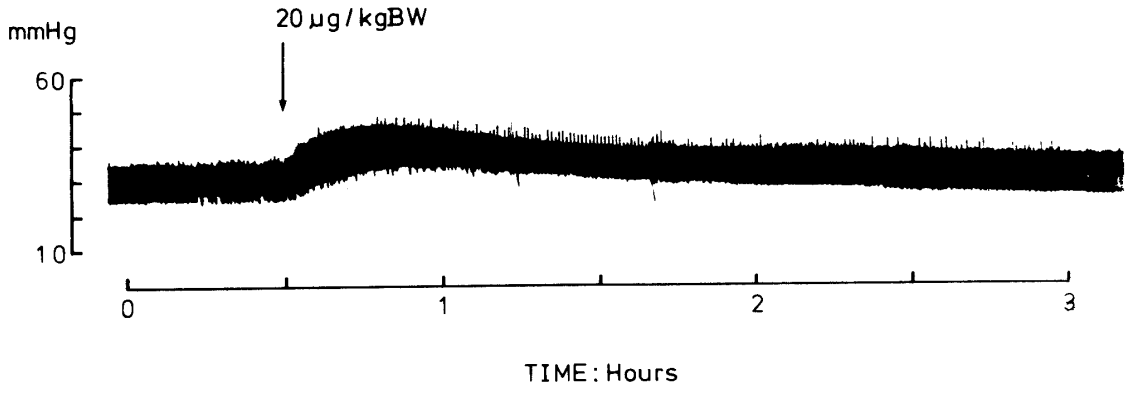

Fig.1. A chart record showing the effect of urophysial extract (UE) of the carp (2×10^{-5} g acetone-dried urophysis per kg BW) on blood pressure measured in the ventral aorta of the carp.

RESULTS

UE of carp induced a vasopressor effect on the carp ventral aorta (Fig. 1). A dose-dependent relationship was obtained (Fig. 2). A significant minimum increase of blood pressure was obtained by a single injection of 6×10^{-7} g/kg BW of UE (n=5). The peak of the response was observed about 15 min after the injection of

lower doses of UE (to 6 x 10^{-6} g/kg BW); after the injection of higher doses, the peak was delayed to about 30 min. The duration of the vasopressor effect varied according to doses, about 1 hr at a low dose (2 x 10^{-7} g/kg BW) and more than 3 hrs at a high dose (2 x 10^{-4} g/kg BW).

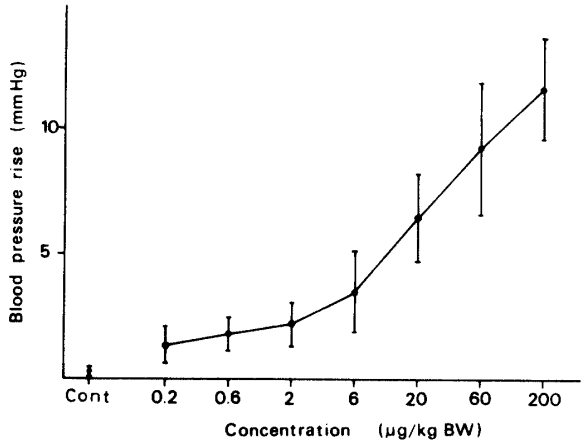

Fig.2. Dose-response curve of the pressor effect of UE in the ventral aorta of the carp. Vertical bars indicate standard errors. Number of experiment was 5.

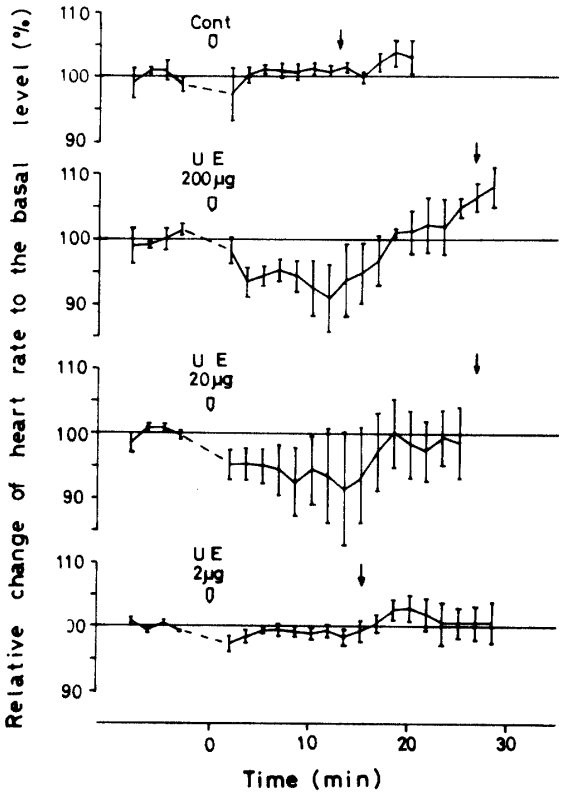

Fig.3. Chronotropic effect of UE in vivo. Arrow indicates the mean of the peaks of blood pressure. White arrow indicates the injection time. The mean heart rate for 8 min before each injection was taken as 100 %. Vertical bars indicate standard errors.

Slight negative chronotropic effects were observed after UE injections (2×10^{-5} g/kg BW - 2×10^{-4} g/kg BW)(Fig. 3). The effects lasted for a short period and the heart rate recovered before the peak of the vasopressor effect. No chronotropic effect was observed after the peak of the vasopressor effect except for the highest dose, where a slight positive chronotropic effect was observed in a few preparations.

The helically cut strip of the ventral aorta showed a contraction in response to UE (Fig. 4). The contraction occured about 1 min after UE addition. A dose-response curve was obtained by plotting maximum contractions induced by different doses of UE

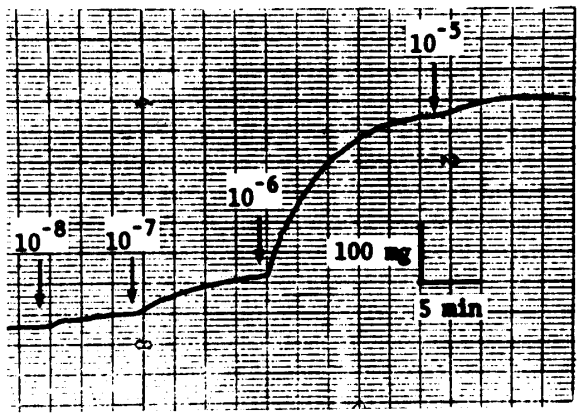

Fig.4. A chart record indicating the responses to cumulatively (10^{-8}-10^{-5} g/ml) applied UE on a ventral aortic strip of the carp.

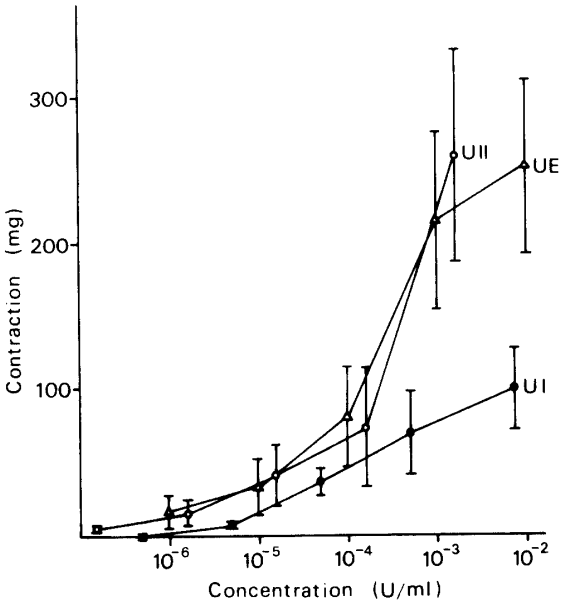

Fig.5. Dose-response curves of the contractile effect of UE, partially purified urotensin I (UI) and urotensin II (UII) in the aortic strip of the carp. Vertical bars indicate standard errors. Number of experiment was 4. One unit of activity is the activity induced by 1 mg acetone-dried carp urophysis.

(Fig. 5). A detectable minimum contraction of the aortic strip was induced by a concentration of 10^{-6} U/ml (10^{-9} g/ml) of UE. The maximum contraction was observed 20 to 25 min after applying 10^{-5} g/ml of UE, and the response continued for more than 1 hr with little decrease. The contraction induced by 10^{-5} g/ml of UE (227.5±22.2 mg) was the same as the maximum response of ACh (10^{-6}-10^{-5} M) (211.0±20.0 mg). Atropin (10^{-9} and 10^{-8} M) and phentolamine (10^{-6} M) did not affect the response to UE on the aortic strip.

Dose-dependent contraction by UII was also observed in the aortic strip (Fig. 5). It was found that contraction by UE was almost entirely due to UII contained in UE (Fig. 5). UI also induced dose-dependent contraction, however, the activity of UI was less than 2% of that of UE in the higher doses(Fig. 5). The time courses of responses in the aortic strip to UII and UI were almost the same as that of UE.

An enhancement of the contractile force of beating of the electrically driven atrial strip was induced by 10^{-5} g/ml of UE (Fig. 6). The enhancement was observed about 2 min after UE addition and the maximum contraction was attained about 20 min after the onset of the response. The mean enhancement of the contractile force of beating induced by 10^{-5} g/ml of UE was 27±7% of the contractile force of beating before the addition. This enhancement was significant ($p<0.03$). The mean enhancement induced by 10^{-6} g/ml of UE was not significant (18±11%).

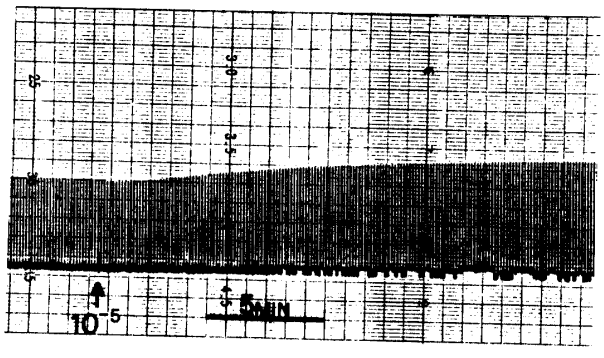

Fig.6. A chart record showing the effect of UE (10^{-5} g/ml) on the contractile force of the electrically driven atrial strip of the carp.

DISCUSSION

The vasopressor effect of UE has already been demonstrated

in the eel (Anguilla anguilla, 3, 5; A. japonica, 6; A. rostrata, 16), but not in other species. In the present study, dose-dependent vasopressor effect of carp UE was demonstrated in the same species. It seems that the vasopressor effect of UE is common in teleosts. A slight negative chronotropic effect of UE was observed in vivo, but the effect had disappeared at the peak period of the vasopressor effect, suggesting that this negative chronotropic effect of UE was a reflex of the rapid increase in blood pressure. Furthermore, the minimal positive chronotropic effect in vivo except for a few preparations in the highest doses suggests that the vasopressor effect of UE is mediated through peripheral vasocontraction or a positive inotropic effect in the heart.

The present investigation demonstrated that UE induced contraction of the isolated ventral aortic strip. This indicates that the vasopressor effect induced by UE is at least partially due to the contraction of the ventral aorta. Furthermore, the significant enhancement of the contractile force of the atrial strip by UE of the carp was demonstrated in the present study. There is the possibility that UE is also effective on the atrium of the carp and that the vasopressor effect induced by UE is partially due to its inotropic effect. The effective dose in the heart is higher than that in the aorta, suggesting that the vasopressor effect is mediated mainly by the vasocontraction on the ventral aorta, and probably on the arterioles.

Recently, 2 kinds of peptides from UE were isolated and determined; urotensin I (UI), 41 residues peptides (17, 18) and urotensin II (UII), 12 residues peptides (19, 20). The present results indicate that the contractile effect of UE is explained by UII in UE.

The UI preparation also elicited contraction of the aortic strip in the present study. Using partially purified UI and UII of Catostomus c., Chan (16) reported that UI as well as UII had a vasopressor effect in the eel. These results indicate the possibility that UI has some effect on the cardiovascular system of fish, although it is necessary to consider the possibility of contamination of UII in the UI preparation. Further experiment using pure UI is necessary to determine the effect of UI on the cardiovascular systems of fish.

The control of the autonomic nervous system on the vascular system in fish is known (21). In the carp, the predominant

cholinergic control and the minor adrenergic control on the ventral aorta have been demonstrated (10). In the present study, it was shown that the maximum contraction induced by ACh was attained by 10^{-5} g/ml of UE, and that the contractile effect of UE on the aortic strip was not inhibited by either phentolamine (alpha-adrenergic blocking agent) or atropin (anticholinergic agent). These results suggest that UE acts directly on the smooth muscle of the aorta, and furthermore, that the contractile action of UE is quite potent.

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