The Journal of Experimental Biology **199**, 1873–1880 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JEB0439

SYNERGISTIC ACTION OF NEUROPEPTIDE Y AND ADRENALINE IN THE EEL ATRIUM

TOSHIHIRO UESAKA

Laboratory of Physiology, Faculty of Integrated Arts and Sciences, Hiroshima University, Higashi-Hiroshima, Hiroshima 739, Japan

Accepted 16 April 1996

Summary

To investigate the influence of neuropeptide Y (NPY) on heart function in a relatively simple model system, the effects of eel NPY (eNPY) on the eel atrium were examined. Eel NPY enhanced the contractile force of the isolated atrium in a dose-dependent manner, without altering the rate of contraction. Although adrenaline also exerts a positive inotropic effect, the effect of eNPY was not blocked by the β_1-adrenoceptor antagonist betaxolol, indicating that eNPY does not act *via* adrenaline release. When eNPY and adrenaline were applied simultaneously, their effects were additive at lower concentrations but not at higher concentrations. The plateau reached at high concentrations suggests that these two regulators act through a common signal transduction process. One candidate for this is an elevation of the concentration of intracellular free Ca^{2+} ($[Ca^{2+}]_i$), since treatment with eNPY or adrenaline enhanced $[Ca^{2+}]_i$, as assessed by fluorescence of Calcium Green-1. The increase in $[Ca^{2+}]_i$ after eNPY and adrenaline treatment is presumably due to Ca^{2+} influx from the external medium, since the effect was greatly reduced in Ca^{2+}-free Ringer's solution and after treatment with verapamil, a Ca^{2+} channel blocker. Although both eNPY and adrenaline enhanced the atrial contractile force, the time courses were different, with the effect of eNPY being gradual and long-lasting, and that of adrenaline being immediate and transient. It is plausible, therefore, that eNPY and catecholamine(s) act synergistically to cause a long-lasting enhancement of contraction force if the two regulators arrive at the atrium simultaneously. The present study also demonstrates that the eel heart contains eNPY.

Key words: eel, neuropeptide Y, catecholamine, atrium, contraction, intracellular Ca^{2+}, extracellular Ca^{2+}, *Anguilla japonica*.

Introduction

Since the discovery of neuropeptide Y (NPY) in porcine brain (Tatemoto, 1982), NPY-like immunoreactivity has been detected in both the central and peripheral nervous system of mammals (reviewed by Dumont *et al.* 1992). High levels of NPY-like immunoreactivity have also been detected in the mammalian heart, especially in neurones innervating the atrial and ventricular myocardium (Gu *et al.* 1984; Dalsgaard *et al.* 1986; Corr *et al.* 1990). In some mammalian cardiac sympathetic nerve endings, NPY has been shown to co-exist with noradrenaline (Gu *et al.* 1984; Allen *et al.* 1985; Sternini and Brecha, 1985; Dalsgaard *et al.* 1986; Corr *et al.* 1990).

However, the effects of NPY on the mammalian heart are controversial. In the right atrium isolated from guinea pig, positive inotropic and chronotropic effects of NPY have been reported by Lundberg *et al.* (1984), while no effects of NPY were seen by Allen *et al.* (1986). NPY was also reported to have no effect on the papillary muscle isolated from cat, guinea pig and rat (Allen *et al.* 1986), on the auricles of human hearts (Franco-Cereceda *et al.* 1987; Michel *et al.* 1989) and on the atria and ventricles of the rat (Scoot *et al.* 1990). In contrast, in perfused whole hearts isolated from rabbit and guinea pig, NPY exerts a negative inotropic effect (Allen *et al.* 1983*b*; Rioux *et al.* 1986). Treatment with NPY produced a similar negative inotropic effect in atrial and ventricular strips isolated from rat (Balasubramaniam *et al.* 1988; Piper *et al.* 1989) and dog (Rigel *et al.* 1989). These inconsistent effects of NPY may be attributed to the complexity of the mammalian heart, which is influenced by a blood supply from the coronary artery. To analyze the direct effect of NPY on the heart, a simpler model is required.

In this study, an isolated eel atrium was used as such a model. The eel atrium can be easily isolated from the heart and beats spontaneously for more than 10 h in normal Ringer's solution (Yasuda *et al.* 1996). Using this preparation, the effects of eel NPY (eNPY) were examined and compared with those of adrenaline. Furthermore, this study also demonstrated the existence of eNPY in eel heart.

Materials and methods

Biological activity in the eel atrium

Japanese eels *Anguilla japonica*, from farmed stock and weighing approximately 220 g, were kept in sea water (20 °C) for more than 1 week. After decapitation, the heart was rapidly excised and the atrium isolated on ice. It was then tied with two cotton threads, one being connected to the bottom of an experimental chamber and the other to a force transducer (type 45196A, Sanei, Tokyo, Japan). The atrium was bathed in Krebs bicarbonate Ringer's solution consisting of (in mmol l^{-1}): 118.5 NaCl, 4.7 KCl, 3.0 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 24.9 $NaHCO_3$ and 10 lactic acid. In Ca^{2+}-free Ringer's solution, 3 mmol l^{-1} $CaCl_2$ was replaced with 4.5 mmol l^{-1} NaCl. The bathing solution (2.0 ml) was bubbled with a 95 % O_2:5 % CO_2 gas mixture (pH 7.4) at room temperature (24–27 °C). After preloading by 5.9 mN, spontaneous isometric contractions were converted into electrical signals by a transducer connected to a strain amplifier (6M82, Sanei) and these were recorded using an electronic polyrecorder (EPR-10B, Toa, Tokyo, Japan). The rate of contraction was measured simultaneously using a tachometer (type 1321, Sanei).

Measurements of intracellular Ca^{2+} levels

The isolated eel atrium was cut into pieces in Ringer's solution and treated with collagenase (1 mg ml^{-1}) in the same solution for 1 h at room temperature. After rinsing with Ringer's solution, the partially dispersed tissue was incubated with Calcium Green-1 acetoxymethyl ester (AM) (5 μmol l^{-1}) and with Pluronic F-127 (0.05 %) as a surfactant for 40 min at room temperature. The atrial cells were stuck onto a glass slide, and this was mounted in a small chamber on the stage of an inverted microscope (Axiovert 135MTV, Zeiss, Tokyo, Japan) and superfused with normal Ringer's solution bubbled with 95 % O_2:5 % CO_2 at room temperature.

The fluorescent signal from Calcium Green-1 was recorded with a laser scanning confocal imaging system (MRC-600, Bio-Rad, Tokyo, Japan; dichroic reflector 510LP; emission filter 515LP) equipped with a Kr–Ar laser (5470K, Ion Laser Technology, Salt Lake City, UT, USA; 488 nm for excitation) and with an inverted microscope (objectives, Zeiss Plan-Neofluar 20×/NA 0.50). The intensity of the fluorescence was expressed on an arbitrary scale of units ranging from 0 to 255.

Isolation of eNPY from heart

The entire heart was removed from 60 eels and the hearts were washed in ice-cold Ringer's solution and stored at –40 °C. A boiled-water extract of eel hearts (91 g wet mass) was prepared following a method described previously (Uesaka *et al.* 1994). The extract was applied to Sep-Pak C18 cartridges (Millipore, Milford, MA). The retained material was eluted with 80 % 2-propanol containing 0.1 % trifluoroacetic acid (TFA), and the eluate was evaporated. The concentrated material was separated by HPLC (LC-6AD system, Shimadzu, Kyoto, Japan) using a C8 reverse-phase column (C8P-50, Asahi Chemical, Kanagawa, Japan). Retained material was eluted with a 90 min linear gradient of 0 % to 90 % acetonitrile containing 10 % 2-propanol and 0.1 % TFA. Fractions with a similar retention time to that of the synthesized eNPY were pooled and further purified by cation-exchange and reverse-phase HPLC. The cation-exchange column (CM-5PW, Tosoh, Tokyo, Japan) was equilibrated with 10 % ethanol and 20 mmol l^{-1} phosphate buffer (pH 6.7). The concentration of NaCl in the eluting solvent was raised to 0.3 mol l^{-1} for the initial 30 min and thereafter to 1.0 mol l^{-1} over 35 min with a linear gradient. In the reverse-phase step (ODS-120T, Tosoh), elution was performed with a linear gradient of 22 % to 32 % acetonitrile containing 10 % 2-propanol and 0.1 % TFA for 50 min. The primary structure of the purified peptide was determined by automated Edman degradation using a gas-phase sequencer (PPSQ-10, Shimadzu).

Reagents

Eel NPY (Tyr-Pro-Pro-Lys-Pro-Glu-Asn-Pro-Gly-Glu-Asp-Ala-Ser-Pro-Glu-Glu-Gln-Ala-Lys-Tyr-Tyr-Thr-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2), which had been isolated from pancreas and intestine of eel (Conlon *et al.* 1991; Uesaka *et al.* 1996), was synthesized automatically using an N^{α}-9-fluorenylmethoxycarbonyl (Fmoc) protection strategy (PSSM-8, Shimadzu) and purified by HPLC on a C18 column (ODS-120T). Drugs used were (–)-adrenaline (Sigma Chemical, St Louis, MO, USA), Calcium Green-1 AM, Pluronic F-127 (Molecular Probes, Eugene, OR, USA), (±)-betaxolol HCl (Mitsubishi Kasei, Tokyo, Japan), collagenase and verapamil HCl (Wako Pure Chemical, Osaka, Japan).

Statistical analyses

Data are reported as mean ± S.E.M.; *N* represents the number of preparations. The statistical significance of differences between means was examined using a Mann–Whitney *U*-test. The null hypothesis was rejected for $P<0.05$.

Results

Effects of eNPY on atrial beating

The isolated atrium from the eel heart continues to beat spontaneously at a constant rate for more than 10 h in normal Ringer's solution. The basal atrial beating force was 1.6±0.1 mN and the rate was 55.7±2.0 beats min^{-1} (N=34). when eNPY (10^{-7} mol l^{-1}) was added to the bathing fluid, the contractile force was enhanced without a significant change in the rate of beating (Fig. 1A). The increase in contractile force was dependent on the concentration of eNPY, with a threshold at 3×10^{-9} mol l^{-1} and a maximal effect at 10^{-6} mol l^{-1} (Fig. 1B).

Table 1 shows the effect of eNPY after blocking adrenaline action with betaxolol, a β_1-adrenoceptor antagonist. In the presence of betaxolol (10^{-5} mol l^{-1}), the force and rate of spontaneous beating tended to be reduced, although the effects were not significant. The effects of adrenaline were completely blocked after treatment with betaxolol (Table 1,

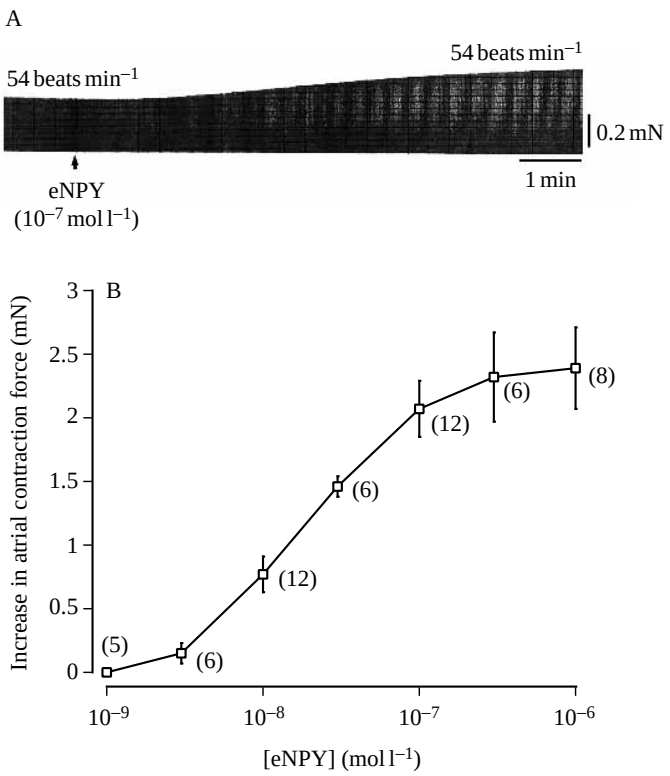

Fig. 1. Effects of eel NPY (eNPY) on eel atrial beating. (A) A representative record of the effects of eNPY (10^{-7} mol l^{-1}). (B) Dose–response curve for the effect of eNPY on the contractile force. The change in the contractile force after addition of eNPY was plotted against its corresponding concentration. Each point and vertical bar indicate the mean value and S.E.M. Numbers of experiments are shown in parentheses.

upper half). Even after blocking adrenaline with betaxolol, eNPY (3×10^{-7} mol l^{-1}) enhanced the contractile force significantly ($P<0.05$).

Interaction between adrenaline and eNPY

The maximal effects of eNPY and adrenaline were then compared (Table 2, upper half). Even at 10^{-6} mol l^{-1}, eNPY had no effect on the beating rate, whereas adrenaline (3×10^{-7} mol l^{-1}) increased it significantly ($P<0.05$). The contractile force was enhanced both by eNPY and by adrenaline. Although the enhancement caused by adrenaline tended to be greater than that produced by eNPY, the difference was not significant. When eNPY and adrenaline were applied simultaneously, the combined effect was not significantly different from that of adrenaline alone. When 10^{-6} mol l^{-1} eNPY was applied after pretreatment with 3×10^{-7} mol l^{-1} adrenaline, further enhancement was not observed (data not shown). These results indicate that there is an upper limit to the effects produced by the combined actions of eNPY and adrenaline. In contrast, when lower concentrations of eNPY (10^{-8} mol l^{-1}) and adrenaline (10^{-8} mol l^{-1}) were applied simultaneously, the increase in contractile force was significantly greater ($P<0.05$) than that induced by adrenaline alone (Table 2, lower half).

Table 1. *Effect of adrenaline and eel neuropeptide Y (eNPY) on the beating of the atrium after pretreatment with betaxolol*

Conditions	Atrial contraction (mN)	Beating rate (beats min^{-1})
Control	1.9±0.3	64.5±2.0
Betaxolol (10^{-5} mol l^{-1})	1.3±0.2	57.7±1.4
Betaxolol (10^{-5} mol l^{-1}) +adrenaline (10^{-7} mol l^{-1})	1.2±0.2	57.6±1.4
Control	1.6±0.3	59.8±2.5
Betaxolol (10^{-5} mol l^{-1})	1.1±0.2	50.4±3.8
Betaxolol (10^{-5} mol l^{-1}) +eNPY (3×10^{-7} mol l^{-1})	2.6±0.3*	50.3±3.9

Values are mean ± S.E.M., $N=6$.
*$P<0.05$ compared with the betaxolol only treatment (Mann–Whitney U-test).

Table 2. *Comparison of the effects of eel neuropeptide Y (eNPY) and adrenaline on the force and frequency of beating of the eel atrium*

Treatment	Increase in atrial beating	
	Force (mN)	Beating rate (beats min^{-1})
eNPY (10^{-6} mol l^{-1})	2.1±0.1	0.4±0.6
Adrenaline (3×10^{-7} mol l^{-1})	2.6±0.3	23.2±7.3*
eNPY (10^{-6} mol l^{-1}) +adrenaline (3×10^{-7} mol l^{-1})	2.9±0.4*	21.3±3.4*
eNPY (10^{-8} mol l^{-1})	1.0±0.3	0.0±0.4
Adrenaline (10^{-8} mol l^{-1})	1.5±0.3	17.0±3.8*
eNPY (10^{-8} mol l^{-1}) +adrenaline (10^{-8} mol l^{-1})	3.0±0.5*,†	18.7±3.0*

The effects of eNPY and adrenaline are shown as the difference between the basal value before treatment and the value after treatment with these regulators.
Values are mean ± S.E.M., $N=6$.
*$P<0.05$ compared with eNPY alone (Mann–Whitney U-test).
†$P<0.05$ compared with adrenaline alone (Mann–Whitney U-test).

Effect of eNPY and adrenaline on the intracellular concentration of free Ca^{2+}

As shown in Fig. 2, both eNPY and adrenaline enhanced the peak concentrations of intracellular free Ca^{2+} ($[Ca^{2+}]_i$) in the eel atrium. After eNPY administration, the peak levels of $[Ca^{2+}]_i$ were enhanced gradually, while they were enhanced transiently after adrenaline application.

To determine whether this Ca^{2+} originates from the extracellular medium or from intracellular stores, Ca^{2+} in the bathing medium was omitted. As shown in Fig. 3A, both the

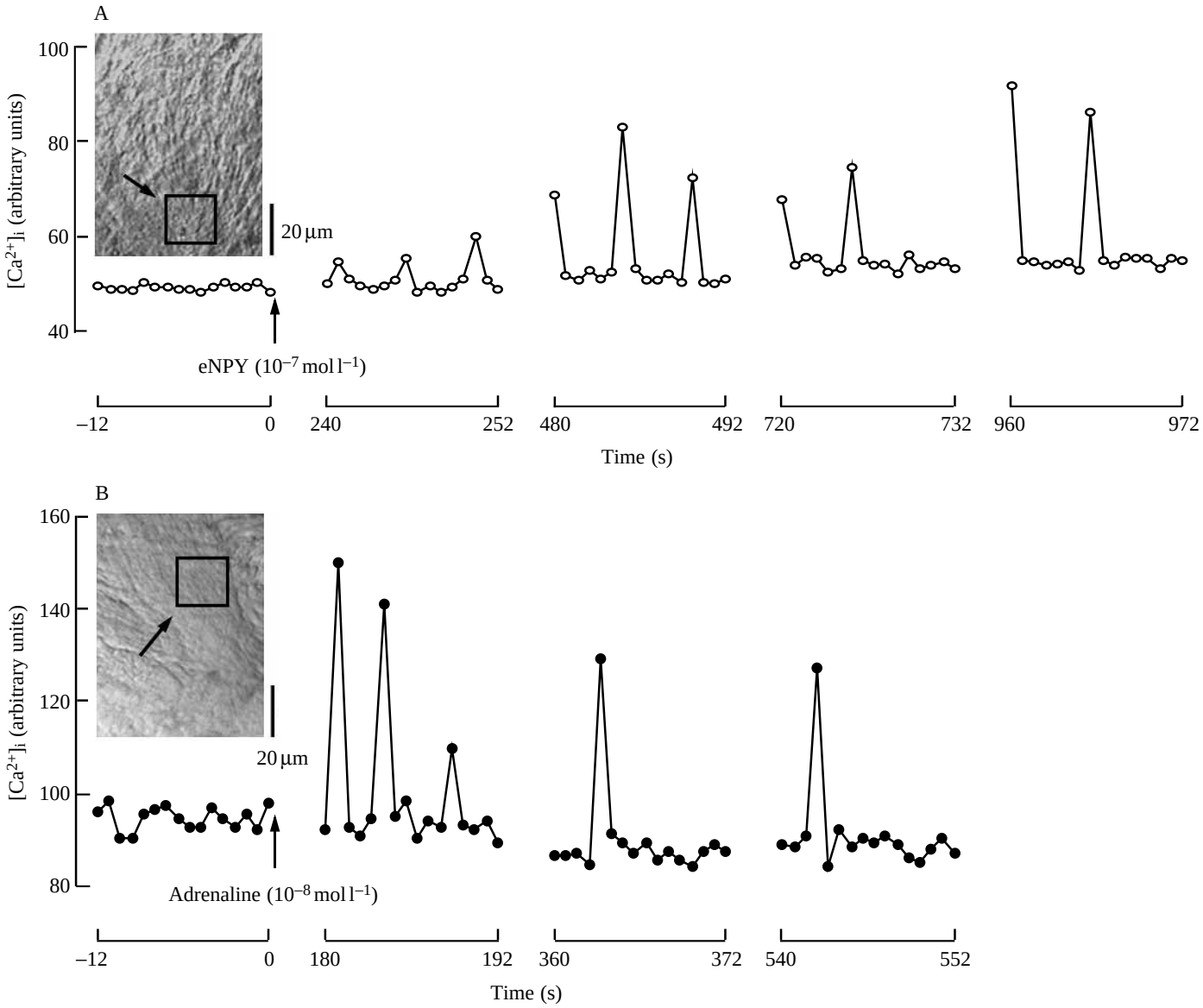

Fig. 2. Oscillation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in a region of the myocardium. The region used to monitor $[Ca^{2+}]_i$ levels is boxed in the phase contrast micrograph inserted into each panel. The brightness of all pixels (N=1200) in the region was counted, and the mean brightness ± S.E.M. was obtained. Error bars were all smaller than the size of the symbol. (A) Time course of $[Ca^{2+}]_i$ in response to eNPY (10^{-7} mol l^{-1}) applied to the myocardium at time zero. (B) Time course of $[Ca^{2+}]_i$ in response to adrenaline (10^{-8} mol l^{-1}) applied at time zero.

contractile force and rate of beating were reduced in the absence of external Ca^{2+}. After reintroducing Ca^{2+} into the bathing solution, both parameters recovered (Fig. 3Ai). In Ca^{2+}-free Ringer's solution, the effect of eNPY was completely abolished (Fig. 3Aii) and that of adrenaline was markedly reduced (Fig. 3Aiii). Similar reductions in the effects of eNPY and adrenaline were also observed in the presence of verapamil (10^{-5} mol l^{-1}), a Ca^{2+} channel blocker (Fig. 3B). After washing out verapamil, however, spontaneous beating did not recover.

Time courses of the effects of eNPY and adrenaline

Using submaximal dosages of eNPY (10^{-7} mol l^{-1}) and adrenaline (3×10^{-8} mol l^{-1}), the time courses of the effects of eNPY and adrenaline on atrial beating were compared (Fig. 4). When adrenaline was added to the bathing fluid, both contractile force and rate of beating increased immediately and maximal effects were obtained after 4–5 min. The contractile force then decreased gradually, i.e. the effect of adrenaline on atrial contractile force was transient. In contrast, eNPY started to enhance the contractile force after 2 min and the effect reached a maximum after 16 min with no change in the rate of beating. When eNPY and adrenaline were applied simultaneously, both the contractile force and the rate of beating increased initially in the same way as in response to adrenaline alone, but the maximal force was maintained for more than 16 min.

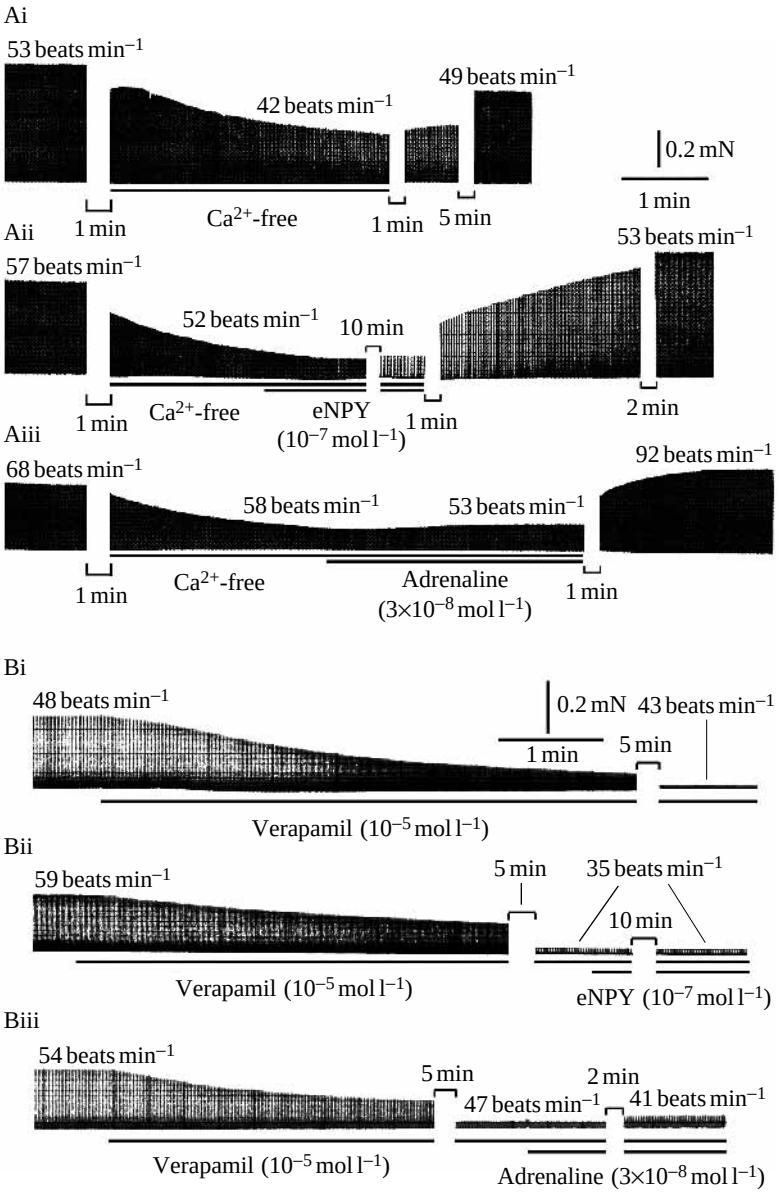

Fig. 3. The effects of extracellular Ca^{2+} on atrial beating in the eel. (A) The effects of eel NPY (eNPY) and adrenaline in the absence of extracellular Ca^{2+}. Ca^{2+} was omitted during the period indicated by the horizontal bar (Ca^{2+}-free). In the absence of external Ca^{2+}, eNPY (10^{-7} mol l^{-1}) or adrenaline (3×10^{-8} mol l^{-1}) was applied during the period indicated by the lower bar (Aii or Aiii). (B) The effects of eNPY and adrenaline in the presence of verapamil. After addition of verapamil (10^{-5} mol l^{-1}), the contractile force and the beating rate diminished gradually (Bi). In the presence of verapamil, eNPY (10^{-7} mol l^{-1}) or adrenaline (3×10^{-8} mol l^{-1}) was applied (lower bar in Bii or Biii).

Isolation of eNPY from an extract of eel heart

To prove that eNPY was present in eel heart, I attempted to isolate eNPY from the heart. Using synthesized eNPY as a marker, a peptide was purified using HPLC. The retention time of the isolated peptide (native) was exactly the same as that of the synthesized eNPY on both reverse-phase and cation-exchange HPLC (Fig. 5Ai,ii, Bi,ii). When a mixture of the native and the synthesized peptides was applied, only a single peak was obtained on both types of HPLC (Fig. 5Aiii, Biii).

The amino acid sequence of the isolated peptide was determined as follows: Tyr-Pro-Pro-Lys-Pro-Glu-Asn-Pro-Gly-Glu-Asp-Ala-Ser-Pro-Glu-Glu-Gln-Ala-Lys-Tyr-Tyr-Thr-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr. This sequence was identical to that of the synthesized eNPY. The isolated peptide increased atrial contractile force without changing the rate of beating (data not shown).

Discussion

Although the role of NPY in the mammalian heart is still obscure, the present study clearly demonstrates that a homologous NPY (eNPY) increases the contractile force of the isolated eel atrium without altering the rate at which the atrium beats. The effect of eNPY was not inhibited by treatment with betaxolol, a β_1-adrenoceptor antagonist (Table 1), indicating that eNPY does not act *via* adrenaline release.

Adrenaline also enhanced atrial contractile force, but the enhancement was not additive to that of a supramaximal dose of eNPY (Table 2). This suggests that the two regulators enhance contractile force through a common process. Enhancement of $[Ca^{2+}]_i$ can be considered as a possible common factor, since eNPY and adrenaline both increase $[Ca^{2+}]_i$ in the eel myocardium (Fig. 2). Similarly, enhancement of $[Ca^{2+}]_i$ by NPY has been reported in many mammalian cells,

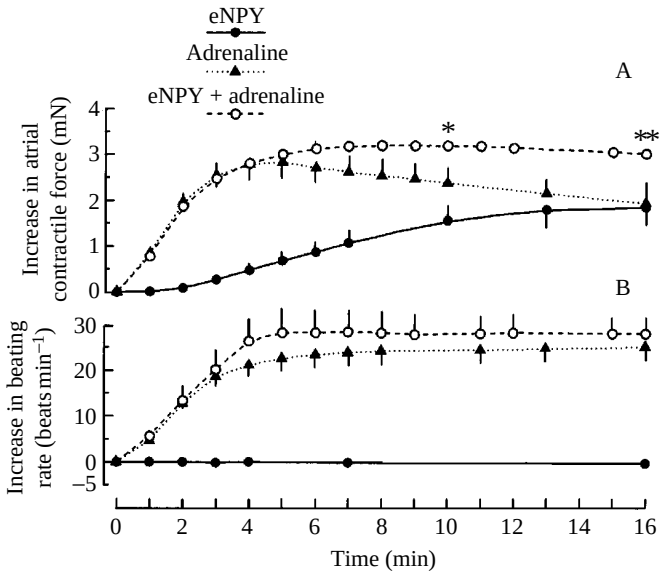

Fig. 4. Time courses of the effects of eel NPY (eNPY) and adrenaline. Changes in the contractile force (A) and the beating rate (B) were plotted against time after the addition of 10^{-7} mol l^{-1} eNPY (●), 3×10^{-8} mol l^{-1} adrenaline (▲) or both agents together (○). All agents were applied to the bathing medium at time zero. Each point and vertical bar indicate the mean value and S.E.M. (N=5). Where no standard error is shown, error bars are smaller than the symbols. *P<0.05, **P<0.01 compared with the effect of adrenaline alone (Mann–Whitney U-test).

including vascular smooth muscle cells (Mihara *et al.* 1989; Wahlestedt *et al.* 1992*b*), dorsal root ganglion (Perney and Miller, 1989), erythroleukaemia cells (Motulsky and Michel, 1988), neuroblastoma cells (Aakerlund *et al.* 1990), adrenal chromaffin cells (Wahlestedt *et al.* 1992*a*) and cells in the ovary (Selbie *et al.* 1995).

Contraction of the eel atrium was dependent on the presence of extracellular Ca^{2+}, and the effects of eNPY and adrenaline were both reduced in Ca^{2+}-free Ringer's solution or by verapamil, a Ca^{2+} channel blocker (Fig. 3). This suggests that the elevation in $[Ca^{2+}]_i$ after eNPY and adrenaline administration is caused by an influx of Ca^{2+} from the extracellular fluid. Extracellular Ca^{2+} has also been implicated in ventricle contraction in frogs (Morad *et al.* 1983; Bers, 1985) and teleosts (Driedzic and Gesser, 1988; Hove-Madsen and Gesser, 1989), whereas intracellular stores of Ca^{2+} are involved in contraction of the mammalian ventricle (see Wier, 1990).

Although both eNPY and adrenaline enhanced the contractility of eel atrium, the time courses of the effects of these regulators were different; the effect of eNPY being gradual and long-lasting, while the effect of adrenaline was immediate and transient (Fig. 4). Since eel heart contains eNPY (as shown in the present study) and catecholamines (Pennec and Le Bras, 1984; Yasuda *et al.* 1996), it is plausible that eNPY and catecholamine(s) act synergistically in the eel atrium. Although at present there is no evidence that eNPY and catecholamine(s) co-exist in the same cell and are co-released in eel heart, there is considerable evidence for the co-existence or co-release of these regulators in mammalian sympathetic nerves (Gu *et al.* 1984; Dalsgaard *et al.* 1986; Corr *et al.* 1990), adrenal gland (Allen *et al.* 1983*a*; Varndell *et al.* 1984; Kuramoto *et al.* 1986) and pancreas (Ahren *et al.* 1989).

I wish to thank Professor Masaaki Ando, Faculty of Integrated Arts and Sciences, Hiroshima University, for his valuable advice and for reading the manuscript and Mr H. Teranishi for the amino acid sequence analysis. This research was supported in part by Grants-in-Aid no. 02804062 from the

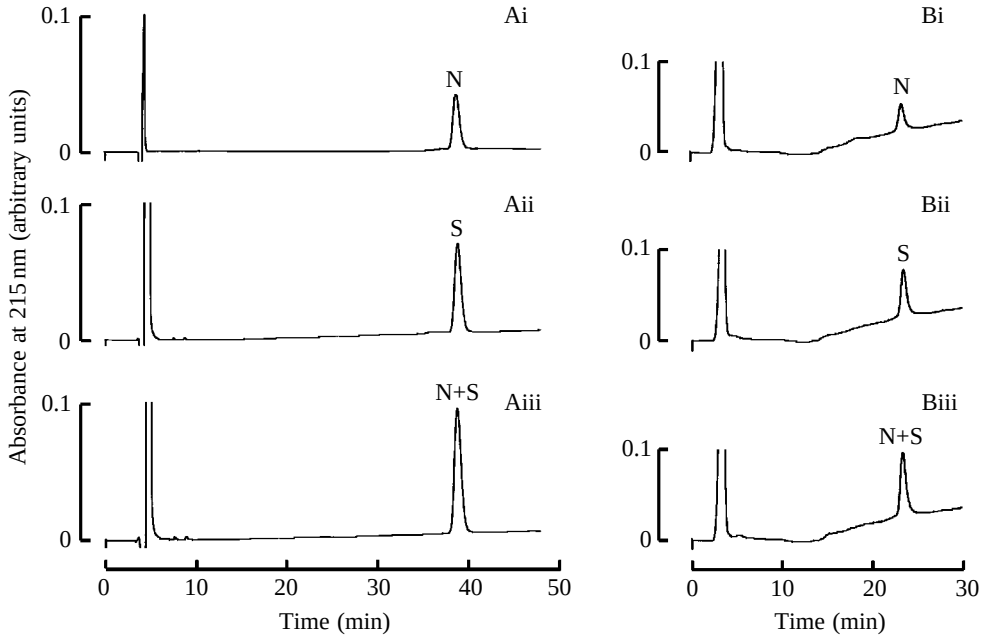

Fig. 5. A comparison of the characteristics of native (N) and synthetic (S) eel neuropeptide Y (eNPY). (A) Reverse-phase chromatograms of N (Ai), S (Aii) and N+S (Aiii) with a TSKgel ODS-120T column. Each peptide alone and a mixture of the two were eluted with a 50 min linear gradient of 22 % to 32 % acetonitrile in 10 % 2-propanol and 0.1 % trifluoroacetic acid. Flow rate was 0.5 ml min^{-1}. (B) Cation-exchange chromatograms with a TSKgel CM-5PW column. N (Bi), S (Bii) and N+S (Biii) were eluted with a 25 min linear gradient of 0 mol l^{-1} to 0.5 mol l^{-1} NaCl in 10 % ethanol and 20 mmol l^{-1} phosphate buffer (pH 6.7). Flow rate was 0.5 ml min^{-1}.

Ministry of Education, Sciences and Culture, Japan, and no. 9233 from the Salt Science Research Foundation, Japan.

References

AAKERLUND, L., GETHER, V., FUHLENDORFF, J., SCHWARTZ, T. W. AND THRASTRUP, O. (1990). Y_1 receptors for neuropeptide Y are coupled to mobilization of intracellular calcium and inhibition of adenylate cyclase. *FEBS Lett.* **260**, 73–78.

AHREN, B., MÄRTEENSSON, H. AND EKMAN, R. (1989). Pancreatic nerve stimulation releases neuropeptide Y – but not galanin – or calcitonin gene-related peptide-like immunoreactivity from the pig pancreas. *J. auton. nerv. Syst.* **27**, 11–16.

ALLEN, J. M., ADRIAN, T. E., POLAK, J. M. AND BLOOM, S. R. (1983*a*). Neuropeptide Y in the adrenal gland. *J. auton. nerv. Syst.* **9**, 559–563.

ALLEN, J. M., BIRCHAM, P. M. M., EDWARDS, A. V., TATEMOTO, K. AND BLOOM, S. R. (1983*b*). Neuropeptide Y (NPY) reduces myocardial perfusion and inhibits the force of contraction of the isolated perfused rabbit heart. *Regul. Pept.* **6**, 247–253.

ALLEN, J. M., GJÖRSTRUP, P., BJÖRKMAN, J. A., EK, L., ABRAHAMSSON, T. AND BLOOM, S. R. (1986). Studies on cardiac distribution and function of neuropeptide Y. *Acta physiol. scand.* **126**, 405–411.

ALLEN, J. M., POLAK, J. M., RODRING, J., DARCY, K. AND BLOOM, S. R. (1985). Localization of neuropeptide Y in nerves of the rat cardiovascular system and the effect of 6-hydroxydopamine. *Cardiovasc. Res.* **19**, 570–577.

BALASUBRAMANIAM, A., GRUPP, I. L., MATTLIB, M. A., BENZA, R., JACKSON, R. L., FISHER, J. E. AND GRUPP, G. (1988). Comparison of the effects of neuropeptide Y (NPY) and 4-norleucine NPY on isolated perfused rat hearts: effects of NPY on atrial and ventricular strips of rat heart and on rabbit heart mitochondria. *Regul. Pept.* **21**, 289–299.

BERS, D. M. (1985). Ca influx and SR Ca release in cardiac muscle activation during poststress recovery. *Am. J. Physiol.* **248**, H366–H381.

CONLON, J. M., BJENNING, C., MOON, T. W., YOUSON, J. H. AND THIM, L. (1991). Neuropeptide Y-related peptides from the pancreas of a teleostean (eel), holostean (bowfin) and elasmobranch (skate) fish. *Peptides* **12**, 221–226.

CORR, L. A., ABERDEEN, J. A., MILNER, P., LINCORN, J. AND BURNSTOCK, G. (1990). Sympathetic and nonsympathetic neuropeptide Y-containing nerves in the rat myocardium and coronary arteries. *Circulation Res.* **66**, 1602–1609.

DALSGAARD, C. J., FRANCO-CERECEDA, A., LUNDBERG, J. M., THEODORSSON-NORHEIM, E. AND HÖKFELT, T. (1986). Distribution and origin of substance P- and neuropeptide Y-immunoreactive nerves in the guinea pig heart. *Cell Tissue Res.* **243**, 477–485.

DRIEDZIC, W. AND GESSER, H. (1988). Differences in force–frequency relationships and calcium dependency between elasmobranch and teleost hearts. *J. exp. Biol.* **140**, 227–241.

DUMONT, Y., MARTEL, J.-C., FOURNIER, A., ST-PIERRE, S. AND QUIRON, R. (1992). Neuropeptide Y and neuropeptide Y receptor subtypes in brain and peripheral tissue. *Progr. Neurobiol.* **38**, 125–167.

FRANCO-CERECEDA, A., BENGTSSON, L. AND LUNDBERG, J. M. (1987). Inotropic effects of calcitonin gene-related peptide, vasoactive intestinal polypeptide and somatostatin on the human right atrium *in vitro*. *Eur. J. Pharmac.* **134**, 69–76.

GU, J., POLAK, J. M., HUANG, W. M., SHEPPARD, M. N., TATEMOTO, K. AND BLOOM, S. R. (1984). High concentrations of a novel peptide, neuropeptide Y, in the innervation of mouse and rat heart. *J. Histochem. Cytochem.* **32**, 467–472.

HOVE-MADSEN, L. AND GESSER, H. (1989). Force–frequency relation in the myocardium of rainbow trout. *J. comp. Physiol.* **159**B, 61–69.

KURAMOTO, H., KONDO, H. AND FUJISAWA, T. (1986). Neuropeptide tyrosine (NPY)-like immunoreactivity in adrenal chromaffin cells and intraadrenal nerve fibers of rats. *Anat. Rec.* **214**, 321–328.

LUNDBERG, J. M., HUA, X. Y. AND FRANCO-CERECEDA, A. (1984). Effects of neuropeptide Y (NPY) on mechanical activity and neurotransmission in the heart, vas deferens and urinary bladder of the guinea pig. *Acta physiol. scand.* **121**, 325–332.

MICHEL, M. C., WIRTH, S. C., ZERKOWSKI, H. R., MAISEL, A. S. AND MOTULSKY, H. J. (1989). Lack of inotropic effects of neuropeptide Y in human myocardium. *J. cardiovasc. Pharmac.* **14**, 919–922.

MIHARA, S. I., SHIGERI, Y. AND FUJIMOTO, M. (1989). Neuropeptide Y induced intracellular Ca^{2+} increases in vascular smooth muscle cells. *FEBS Lett.* **259**, 79–82.

MORAD, M., GOLDMAN, Y. E. AND TRENTHAM, D. R. (1983). Rapid inactivation of Ca^{2+}-antagonists shows that Ca^{2+} entry activates contraction in frog heart. *Nature* **304**, 635–638.

MOTULSKY, H. J. AND MICHEL, M. C. (1988). Neuropeptide Y mobilizes Ca^{2+} and inhibits adenylate cyclase in human erythroleukemia cells. *Am. J. Physiol.* **255**, E880–E885.

PENNEC, J. P. AND LE BRAS, Y. M. (1984). Storage and release of catecholamines by nerve endings in the isolated heart of the eel (*Anguilla anguilla*). *Comp. Biochem. Physiol.* C **77**, 167–171.

PERNEY, T. M. AND MILLER, R. J. (1989). Two different G-proteins mediate neuropeptide Y and bradykinin-stimulated phospholipid breakdown in cultured sensory neurons. *J. biol. Chem.* **264**, 7317–7327.

PIPER, H. M., MILLAR, B. C. AND MCDERMOTT, B. J. (1989). The negative inotropic effect of neuropeptide Y on the ventricular cardiomyocyte. *Naunyn-Schiedeberg's Arch. Pharmac.* **340**, 333–337.

RIGEL, D. F., GRUPP, I. L., BALASUBRAMANIAM, A. AND GRUPP, G. (1989). Contractile effects of cardiac neuropeptides in isolated canine atrial and ventricular muscles. *Am. J. Physiol.* **257**, H1082–H1087.

RIOUX, F., BACHELARD, H., MARTEL, J. C. AND ST-PIERRE, S. (1986). The vasoconstrictor effect of neuropeptide Y and related peptides in the guinea pig isolated heart. *Peptides* **7**, 27–31.

SCOOT, N. A., MICHEL, M. C., BOUBLIK, J. H., RIVIER, J. E., MOTOMURA, S., CRUM, R. L., LANDON, M. AND BRAWN, M. R. (1990). Distinction of NPY receptors *in vitro* and *in vivo*. Differential effects of NPY and NPY-(18–36). *Am. J. Physiol.* **259**, H174–H180.

SELBIE, L. A., DARBY, K., SCHMITZ-PEIFFER, C., BROWNE, C. L., HERZONG, H., SHINE, J. AND BIDEN, T. J. (1995). Synergistic interaction of Y1-neuropeptide Y and α_{1b}-adrenergic receptors in the regulation of phospholipase C, protein kinase C and arachidonic acid production. *J. biol. Chem.* **270**, 11789–11796.

STERNINI, C. AND BRECHA, N. (1985). Distribution and colocalization of neuropeptide Y and tyrosine hydroxylase-like immunoreactivity in the guinea pig heart. *Cell Tissue Res.* **241**, 93–102.

TATEMOTO, K. (1982). Neuropeptide Y: Complete amino acid sequence of the brain peptide. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5485–5489.

UESAKA, T., YANO, K., YAMASAKI, M. AND ANDO, M. (1996). Effects of eel neuropeptide Y on ion transport across the seawater eel intestine. *Zool. Sci.* (in press).

UESAKA, T., YANO, K., YAMASAKI, M., NAGASHIMA, K. AND ANDO,

M. (1994). Somatostatin-related peptides isolated from the eel gut: Effects on ion and water absorption across the intestine of the seawater eel. *J. exp. Biol.* **188**, 205–216.

VARNDELL, I. M., POLAK, J. M., TERENGHI, G. AND BLOOM, S. R. (1984). Neuropeptide tyrosine (NPY) immunoreactivity in norepinephrine-containing cells and nerves of the mammalian adrenal gland. *Endocrinology* **114**, 1460–1462.

WAHLESTEDT, C., REGUNATHAN, S. AND REIS, D. J. (1992*a*). Identification of cultured cells selectively expressing Y1, Y2, or Y3-type receptors for neuropeptide Y/peptide YY. *Life Sci.* **50**, PL7–PL12.

WAHLESTEDT, C., REIS, D. J., YOO, H., ANDERSSON, D. AND EDVINSSON, L. (1992*b*). A novel inositol phosphate selectively inhibits vasoconstriction evoked by the sympathetic co-transmitters neuropeptide Y (NPY) and adenosine triphosphate (ATP). *Neurosci. Lett.* **143**, 123–126.

WIER, W. G. (1990). Cytoplasmic $[Ca^{2+}]_i$ in mammalian ventricle: Dynamic control by cellular processes. *A. Rev. Physiol.* **52**, 467–485.

YASUDA, M., UESAKA, T., FURUKAWA, Y. AND ANDO, M. (1996). Regulation of atrial contraction in the seawater-adapted eel, *Anguilla japonica*. *Comp. Biochem. Physiol.* (in press).