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Natriuretic peptides and the control of catecholamine release in two freshwater teleost and a marine elasmobranch fish

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Abstract

Experiments were carried out *in situ* and *in vivo* to investigate the relationship between natriuretic peptides (NPs) and humoral catecholamine secretion in the American eel (*Anguilla rostrata*), rainbow trout (*Oncorhynchus mykiss*) and spiny dogfish (*Squalus acanthias*). *In situ* perfusion of the chromaffin tissue of *A. rostrata* with homologous atrial NP (ANP; 10^{-9} mol l^{-1}) or ventricular NP (VNP; 10^{-9} mol l^{-1}), or *O. mykiss* with either rat ANP (10^{-9} mol l^{-1}), eel VNP (10^{-9} mol l^{-1}), or trout VNP (10^{-9} mol l^{-1}), did not significantly affect basal or carbachol-elicited (10^{-5} mol kg^{-1}) catecholamine release. Bolus injections of homologous ANP (10^{-9} mol kg^{-1}) or VNP (10^{-9} mol kg^{-1}) in *A. rostrata in vivo* elicited a rapid and prolonged reduction in arterial blood pressure and an increase in heart rate (f_H); circulating plasma catecholamine levels were unaffected. In *O. mykiss*, bolus injections of rat ANP (10^{-9} mol kg^{-1}) or trout VNP (10^{-9} mol kg^{-1}) elicited a significant bi-phasic pressor-depressor response and a marked increase in f_H. Neither the acute pressor or the longer-term depressor effects of NPs in *O. mykiss* were associated with any change in circulating plasma catecholamine levels. In *S. acanthias*, bolus injections of homologous C-type natriuretic peptide (CNP; 10^{-9} mol kg^{-1}) elicited a bi-phasic pressor-depressor response, an increase in systemic resistance, a decrease in cardiac output and stroke volume, but no change in f_H. Plasma noradrenaline levels were elevated 15-fold after CNP injection while circulating adrenaline levels remained unchanged. These results show that NPs of systemic origin do not directly or indirectly affect basal or cholinergically-mediated catecholamine release in *A. rostrata* and *O. mykiss* and that the initial pressor response to NP's in trout cannot be attributed to an elevation of circulating catecholamines. Conversely, CNP appears to be a potent secretagogue (direct or indirect) of noradrenaline release in *S. acanthias* and thus there is likely to be a significant humoral adrenergic component to the cardiovascular effects of NPs in this species.

Introduction

In fish, catecholamines function as either neurotransmitters or hormones in the circulation (Nilsson 1984). The catecholamines that enter the circulation are synthesized, stored, and released from chromaffin cells primarily embedded in the walls of the posterior cardinal vein in the region of the head kidney in teleosts and in the axillary bodies in elasmobranchs (Nandi 1961; Nilsson 1984; Reid et al. 1995). Cholinergic stimulation of these chromaffin cells via preganglionic sympathetic nerve fibers is thought to be the primary control mechanism of catecholamine secretion (Nilsson 1984). However, in both fish and mammals, there is an extensive list of secondary bioactive substances that modulate, or act independently of, acetylcholine-stimulated catecholamine release (Burgoyne 1991; Reid et al. 1998).

In mammals, natriuretic peptides (NPs) are known to modulate catecholamine release (Kuchel et al. 1987). These hormones, which are produced by the heart, central nervous system, and the adrenal medullary cells (Forssmann et al. 1989), act on the vasculature, kidneys, and adrenals to reduce systemic blood pressure and intravascular volume (Brenner et al. 1990). The vasodepressor activity of NPs in mammals is accomplished, in part, by an inhibition of catecholamine secretion (Drewett et al. 1988; Vatta et al. 1994). Perfusion of bovine adrenal medulla with atrial natriuretic peptide (ANP; 10^{-8} mol l^{-1}) inhibits the release of catecholamines induced by depolarizing solutions of KCl, acetylcholine, or angiotensin II (Fernandez et al. 1992). Similarly, ANP (10^{-8} mol l^{-1}) inhibits both spontaneous and KCl-evoked noradrenaline release in rat adrenal medulla (Vatta et al. 1993). Intravenous injections of ANP in humans were reported to decrease plasma adrenaline levels (Richards et al. 1985).

NPs also are potent vasodilators in both teleosts and elasmobranchs (see Olson 1992; Takei and Balment 1993 for reviews). The presence of ANP binding sites and ANP-immunoreactivity in the chromaffin tissue of different teleosts (Kloas et al. 1994; Wolfensberger et al. 1995) suggest the possibility of an interaction between NPs and catecholamine secretion in fish. In contrast, however, to the inhibitory effects of NPs on catecholamine secretion reported in mammals, perifusion of *Cyprinus carpio* head kidney slices *in vitro* with rat ANP (10^{-7}mol l^{-1}) increased acetylcholine-stimulated adrenaline release (Kloas et al. 1994). The ability of α-adrenoceptor antagonists to inhibit the initial hypertensive effects of bolus injections of NPs in trout (Olson and Duff 1992; Takei et al. 1994a), is further indirect evidence that NPs may stimulate catecholamine release from the chromaffin tissue of fish.

In recent years, the NPs from several teleost and elasmobranch species were isolated and sequenced (see Hagiwara et al. 1995 for review). In both eels □□(Takei et al. 1989, 1990, 1994b) and dogfish □□(Bjenning et al. 1992), discrepancies between the vasodepressor activity of homologous versus heterologous NPs have highlighted the importance of using homologous NPs in physiological studies. The use of sensitive radioimmunoassays has demonstrated that while both ANP and ventricular NP (VNP) are major circulating NPs in eels (Takei et al. 1992, 1994b), C-type natriuretic peptide (CNP) may be the only circulating NP in dogfish (Suzuki et al. 1994).

In view of these findings, we investigated the potential interactions between homologous or heterologous NPs and catecholamine secretion using ANP or VNP in American eels (*Anguilla rostrata*) and rainbow trout (*Oncorhynchus mykiss*), and CNP in spiny dogfish (*Squalus acanthias*). Specifically, using *in situ* posterior cardinal vein perfusion preparations of *A. rostrata* and *O. mykiss*, we investigated the direct effects of NPs on basal catecholamine release and the potential modulatory effects of NPs on the cholinergic control of catecholamine release. *In vivo*, the systemic effects of bolus injections of NPs on catecholamine release were investigated in all three species. In rainbow trout, we also investigated the acute effects of bolus injections of NPs on catecholamine release to determine if the previously described initial pressor effect of NPs (see above for references) could be attributed to elevated circulating catecholamine levels. In the *in vivo* experiments, the relative bioactivity of the NP injections was also assessed by simultaneously monitoring their cardiovascular effects.

Materials and methods

Experimental animals

American eels of either sex weighing between 268 and 1077 g (experimental *N*=47) were obtained from a local commercial supplier (Lancaster, Ontario) and were transported on ice to the University of Ottawa. Rainbow trout of either sex weighing between 190 and 938 g (experimental *n*=58) were obtained from Linwood Acres Trout Farm (Cambellcroft, Ontario) and were transported in oxygenated water to the University of Ottawa.

Trout and eels were maintained on a 12L:12D photoperiod in 1300 l fiberglass tanks supplied with flowing, aerated and dechlorinated City of Ottawa tap water (14 °C). They were allowed at least 4 weeks to acclimate to the holding conditions before experimentation. While trout were fed a commercial diet of Purina Trout Chow, eels were not fed.

Pacific spiny dogfish of either sex weighing between 1315 and 2160 g (experimental *n*=12) were collected by net during trawls by local fishermen and transported to holding facilities at Bamfield Marine Station (Bamfield, British Columbia). The dogfish were kept under natural photoperiod in a 75000 l opaque circular tank provided with aerated full-strength sea water at 11 °C. They were fed twice

weekly with herring and used for experimentation within 4 weeks of their capture.

In situ *experiments*

To investigate the tissue-specific effects of natriuretic peptides (NPs) on catecholamine release at the level of the chromaffin cells, and the potential modulatory role of NPs on catecholamine release elicited by cholinergic stimulation, an *in situ* posterior cardinal vein (PCV) preparation (Reid and Perry 1994) was employed with the following modifications. Rainbow trout and American eels, killed with an overdose of anaesthetic (15 ml l^{-1} 2-phenoxy-ethanol, Sigma Chemical Co., St. Louis, Missouri, USA), were placed on their back in a cradle of ice. An incision was made from the vent to the pectoral girdle and the internal organs and connective tissue were pushed aside in order to cannulate the PCV and the bulbus arteriosus. An inflow cannula (PE 160) was inserted into the PCV approximately two-thirds along the length of the kidney in the anterograde direction. The body cavity was filled with lint-free wipes, and a ligature was placed around the entire fish to secure the inflow cannula. In the trout, an outflow cannula (PE 160) was inserted through the bulbus and into the ventricle. In eels, however, the cannula was not fed through to the ventricle. In each case, the outflow cannula was secured with a ligature around the walls of the bulbus. The head kidney was then perfused with aerated modified Cortland (Wolf 1963) saline (trout: pH=7.8, eels: pH=8.0) according to the basic method of Reid and Perry (1994). Two 1 l beakers, one containing saline, the other containing either saline (control) or a NP saline solution (10^{-9}mol l^{-1}), were aerated and embedded in crushed ice approximately 30 cm above the animal. The positive pressure difference between the surface of the source perfusate and the outflow cannula provided for a perfusion flow rate of $1-2$ ml min^{-1}. The preparations were perfused for 21 min before commencing an experiment. Experiments were initiated by collecting the outflow perfusate in pre-weighed 1.5-ml centrifuge tubes while recording filling time. Pre- and post-sampling weights were divided by filling time to determine perfusate flow rate. After three control samples were collected at 1-min intervals (C1, C2 and C3), the source perfusate was switched from pure saline to either saline containing a natriuretic peptide or to a second beaker of pure saline; perfusate was collected at 1, 2, 3, 4, 5, 7.5, 10, 12.5, and 15 min after the switch. The preparation was then given a bolus injection (300 μl) of the cholinergic agonist carbachol (10^{-5} mol kg^{-1}; Research Biochemical International, Natick, MA), while continuously being perfused with the natriuretic peptide solution, by way of a three-way valve over the course of 1 min and the perfusate was collected each minute for another 5 min (17, 18, 19, 20, 21 min). Triton-X 305 (Sigma) detergent (10 μl l^{-1}) was added to all saline used in these experiments to prevent the NPs from adhering to the tubing before entering the animal. Perfusate samples were immediately placed in liquid nitrogen and later stored at -80 °C until they could be analyzed for catecholamine content. In the American eel, the *in situ* head kidney preparation was used to assess the effects of saline, eel ANP (eANP; 10^{-9} mol l^{-1}), and eel VNP (eVNP; 10^{-9} mol l^{-1}) on both basal and carbachol-elicited catecholamine release. In the rainbow trout, the *in situ* preparation was used to assess the effects of saline, rat ANP (rANP), eVNP, and trout VNP (tVNP) on basal and carbachol-elicited catecholamine release; all NP's were used at a concentration of 10^{-9} mol l^{-1}.

In vivo *experiments*

Surgical procedures

The caudal vein and caudal artery were cannulated in American eel, rainbow trout, and spiny dogfish to permit injection of NPs and repeated blood sampling via the vein, as well as the monitoring of blood pressure (P_{CA}) and heart rate (f_H) via the artery.

Eels were immersed in a buffered ($NaHCO_3$; 4 g l^{-1}) anaesthetic solution of ethyl-*m*-aminobenzoate (2 g l^{-1}; MS-222; Syndel, Vancouver, BC) for approximately 8 min. They were then placed on a dissection tray without gill irrigation. An incision was made immediately below, and parallel to, the lateral line in the caudal region, 2/3 of the way between the head and the tail. Muscle and connective tissue were cleared away allowing for the removal of two spines, exposing the caudal artery (dorsal) and caudal vein (ventral). These vessels were freed from the surrounding vertebrae and connective tissue and occlusively cannulated in the anterograde direction with polyethylene tubing (PE 50; Clay Adams® , Becton Dickinson, Sparks, MD) filled with heparinized (50 iu ml^{-1} sodium heparin; Sigma) teleost Cortland saline. The free ends of the cannulae were threaded through small holes punctured in the skin immediately posterior to the incision. The incision was closed with a running stitch and the protruding cannulae were secured to the side of the eel with silk ligatures.

Trout were immersed in an oxygenated buffered ($NaHCO_3$; 0.2 g l^{-1}) anaesthetic solution of ethyl-*m*-aminobenzoate (0.1 g l^{-1}; MS-222; Syndel) until breathing movements stopped. The fish were then placed on an operating table where their gills were force-ventilated with the same anaesthetic solution. A lateral incision was made in the caudal peduncle to expose, separate from the surrounding tissue, and cannulate (PE 50; Clay Adams) the caudal vein in the anterograde direction. In addition, the caudal artery was cannulated (PE 50) with a blind puncture in the anterograde direction approximately 2 mm dorsal to the caudal vein. The remainder of the procedure was the same as in the eel.

Spiny dogfish were immersed in an aerated anaesthetic solution of ethyl-*m*-aminobenzoate (0.1 g l^{-1}; MS-222; Syndel) and transferred to an operating table where the gills were irrigated continuously with the same anaesthetic solution. A lateral incision was made in the caudal peduncle to expose, separate from the surrounding tissue, and cannulate (PE 50; Clay Adams) both the caudal vein and the caudal artery in the anterograde direction. The cannulae were filled with heparinized (100 i.u. ml^{-1} sodium heparin; Sigma) dogfish saline (500 mmol l^{-1} NaCl). In addition, the pericardial cavity was exposed with a ventral midline incision and the pericardium was dissected to expose the conus arteriosus. To allow measurement of cardiac output (Q), a 3S or 4S ultrasonic flow probe (Transonic Systems Inc., Ithaca, NY) was placed non-occlusively around the conus. Lubricating jelly was used with the perivascular flowprobe as an acoustic couplant. Silk sutures were used to close the ventral and caudal peduncle incisions, and to anchor the cardiac output probe lead and the cannulae to the skin. Following surgery, eels, trout, and dogfish were placed into individual flow-through opaque acrylic or wood (dogfish only) boxes and left to recover for 24 hours before experimentation.

Experimental protocol

Upon establishing stable baseline blood pressure for at least one hour, the effects of a bolus injection (400 μl) of either saline or NP (10^{-9} mol kg^{-1}) on plasma catecholamines and cardiovascular parameters were investigated. The effects of saline, eANP, and eVNP were tested in the American eel; saline, rANP, and tVNP in rainbow trout; saline and dogfish CNP (dfCNP) in the spiny dogfish. The bolus injection was administered by way of the venous cannula and was followed by 0.2 ml of saline. The cardiovascular responses to the injection were monitored continuously over a period of 6 h in the eel and trout, and for 50 min in dogfish. Blood samples (400 μl) were taken at predetermined intervals through the venous cannula and replaced by an equivalent volume of saline. In the eel and trout experiments, the blood samples were drawn 10 min prior to injection (control) and at 5, 10, 30, 60, 120, 180 and 360 min after injection. In the dogfish, blood samples were withdrawn 10 min prior to injection (control) and at 5, 10, 15, 20, 25, 30, and 40 min after injection. All blood samples were collected in 1.5 ml tubes and centrifuged immediately at 10000 g for 15 s. The plasma was decanted, frozen in liquid nitrogen and stored at -80°C for later analysis of catecholamines.

Additional experiments were performed to investigate the possible involvement of plasma catecholamines in the previously described α-adrenergic pressor response that immediately follows a bolus injection of NP in rainbow trout (Olson and Duff 1992; Takei et al. 1994a; Olson et al. 1997). During these acute experiments, blood samples (400 μl) for catecholamine analysis were drawn 10 min prior to injection (control), as well as 1.5, 3, 5, 10, and 15 min after the bolus injection (400 μl) of either saline, rANP (10^{-9}mol kg^{-1}), or tVNP (10^{-9} mol kg^{-1}). Again, cardiovascular parameters were continuously monitored. The sampling procedure and handling of samples were performed as described above.

Analytical techniques

Plasma and perfusate noradrenaline and adrenaline levels were determined on alumina-extracted samples (200 μl) using high pressure liquid chromatography (HPLC) with electrochemical detection (Bernier and Perry 1997). The HPLC consisted of a Varian Star 9012 solvent delivery system (Varian Chromatography Systems, Walnut Creek, CA) coupled to a Princeton Applied Research 400 electrochemical detector (EG&G Instruments, Princeton, NJ). The extracted samples were passed through an Ultratechsphere ODS-C_{18}5 μm column (HPLC Technology Ltd., Macclesfield, U.K.) and the separated amines were integrated with the Star Chromatography software program (version 4.0, Varian). Concentrations were calculated relative to appropriate standards and with 3,4-dihydroxybenzylamine hydrobromide (DHBA) as an internal standard in all determinations.

Caudal artery blood pressure (P_{CA}) was measured with a UFI model 1050BP (UFI, Morro Bay, CA)

pressure transducer that was calibrated against a static water column. Mean blood pressure was calculated as: (systolic pressure + diastolic pressure)/2. The perivascular flow probes used to measure Q in dogfish were connected to a small animal blood flow meter (Transonic Systems, Inc., Ithaca, NY). These probes were pre-calibrated in the factory and verified in the laboratory by pump perfusion of the heart of an immersed euthanized fish with saline at known flow rates. Both P_{CA} and Q signals were recorded with a data acquisition system (Biopac System Inc., Goleta, CA) and collected at 0.04 sec intervals using Acknowledge III^{TM} (Biopac System Inc.) data acquisition software. Systemic vascular resistance (R_S) was calculated as mean P_{CA} divided by Q (i.e. $R_S = P_{CA} / Q$), f_H was derived from the dorsal aortic pressure pulse trace, and stroke volume (V_S) was calculated as Q divided by f_H (i.e. $V_S = Q / f_H$).

Chemicals

Eel ANP, eel VNP, trout VNP, and dogfish CNP-22 were generously provided by the Peptide Institute, Protein Research Foundation (Osaka, Japan); and rat ANP (Ile-26) by E. Blaine, Dalton Cardiovascular Center, University of Missouri (Columbia, MO). Approximate physiological doses of NPs (*in situ*: 1 nmol/l, *in vivo*: 1 nmol/kg) were determined from results obtained by Kaiya and Takei (1996a) using a homologous radioimmunoassay.

Statistical analysis

Data are presented as mean values ± one standard error of the mean (S.E.M.). In both the *in situ* and *in vivo* experiments, the statistical significance of the observed effects of a given treatment over time were tested using a one-way repeated measures analysis of variance (ANOVA). Dunnett's *post-hoc* multiple-comparison test was used to compare the pre-injection control data point (C3) with values at subsequent and previous (C1 and C2) times. To prevent the high variance of the catecholamine data following the injection of carbachol in the *in situ* experiments from masking any changes in basal secretion prior to the injection, separate one-way repeated measures ANOVAs were performed with the -3 to 12.5 min data and the 15 to 21 min data. The statistical significance of observed differences between the *in situ* treatments at a given time was tested using a one-way ANOVA. Dunnett's *post-hoc* multiple-comparison test was used to determine which group(s) differed from the control (saline) group. In order to reduce some of the variability resulting from the long-term temporal differences in the response of individual fish to NP injections, the P_{CA} and f_H data from the eel and trout long-term (6 h) *in vivo* treatments were standardized to the mean value 20 min before injection. The significance level of all statistical tests was $P<0.05$.

Results

In situ *experiments*

Perfusion of PCV preparations of American eel with saline, eANP, or eVNP did not affect the basal secretion rate of either noradrenaline (Figure 1A; C1 to 12.5 min) or adrenaline (Figure 1B; C1 to 12.5 min) over time. After the injection of carbachol, with a single exception (t = 21 min; Figure 1B), the noradrenaline (Figure 1A) and adrenaline (Figure 1B) secretion rates were increased significantly over the final 5 samples in each of the three treatments (17 to 21 min). With the exception of a single sampling time (t = 7.5 min; Figure 1A), there were no significant differences in either the noradrenaline (Figure 1A) or adrenaline (Figure 1B) secretion rates between the saline, eANP, and eVNP treatments at any single sampling points.

Perfusion of PCV preparations of rainbow trout with either saline, rANP, eVNP, or tVNP did not significantly affect the basal secretion rate of noradrenaline (Figure 2A; C1 to 12.5 min) or adrenaline (Figure 2B; C1 to 12.5 min) in the three experimental treatments with respect to control (saline) values at any given sampling point. In all treatment groups, there was a tendency for catecholamine secretion rates to decline over time. Although the extent of the decline differed among the different treatment groups, at any single sampling time, there were no significant differences in the noradrenaline or adrenaline secretion rates between the saline, rANP, eVNP, and tVNP treatments. After the injection of carbachol, the noradrenaline (Figure 2A) and adrenaline (Figure 2B) secretion rates increased significantly and to a similar extent over the final 5 samples in each of the four treatments (17 to 21 min).

In vivo *experiments*

In the Americal eel, bolus injections (10^{-9} mol kg^{-1}) of either eANP or eVNP elicited significant decreases in P_{CA} that developed 15 and 10 min post-injection,

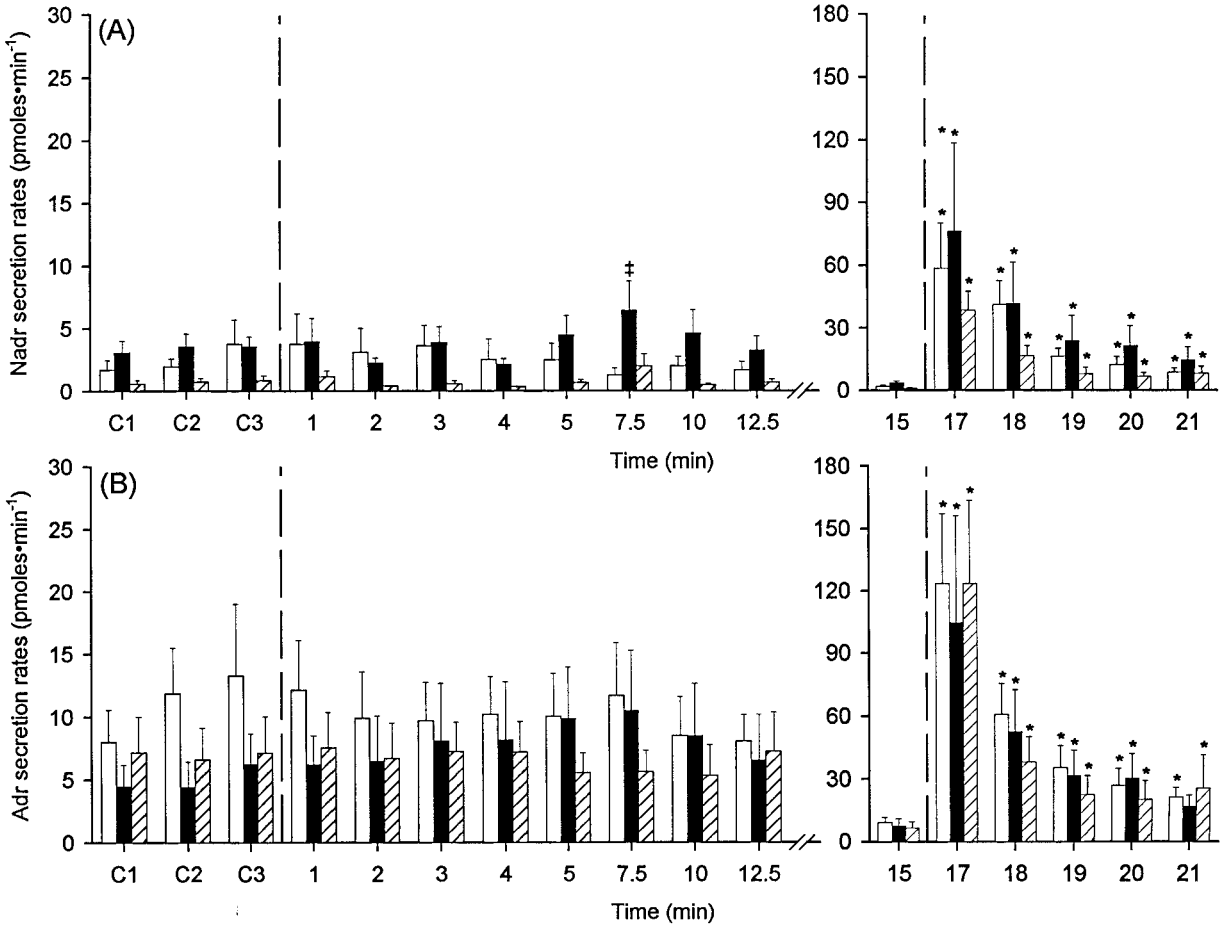

Figure 1. In situ effects of saline (open bars; *N*=10), eel ANP (10^{-9} mol l^{-1}; filled bars; *N*=6), and eel VNP (10^{-9} mol l^{-1}; hatched bars; *N*=6) on basal and carbachol-elicited noradrenaline (Nadr; A) and adrenaline (Adr; B) secretion rates in perfused posterior cardinal vein preparations of American eel, *Anguilla rostrata*. The dashed line after C3 (control 3) indicates the switch from saline to the treatments listed above. The dashed line after 15 min indicates when a bolus injection of carbachol (10^{-5} mol kg^{-1}) was given to the preparations. A ‡ symbol denotes a significant difference from the saline value at any given time. An asterisk denotes a significant difference from the 15 min value for any given treatment ($p<0.05$). Values are means ± 1 SEM.

respectively (Figures 3 and 3C). In both treatments, P_{CA} recovered to control conditions by 6 hours post-injection. The control pre-injection P_{CA} values were 19.1±0.6 and 18.8±1.2 mm Hg for the eANP and eVNP treatments, respectively. The hypotensive effect of NP injection was accompanied by concurrent significant increases in f_H (Figures 3B and 3D). The control pre-injection f_H values were 34.0±4.5 and 29.1±1.2 beats min^{-1} for the eANP and eVNP treatments, respectively. In the control group, saline injection had no significant effect on either P_{CA} (Figure 3E) or f_H (Figure 3F); the control pre-injection P_{CA} and f_H values were 18.6±0.7 mm Hg and 31.6±2.7 beats min^{-1}, respectively. Bolus injections of saline, eANP, or eVNP, were without effect on the plasma concentration of noradrenaline or adrenaline (Table 1). In all treatment groups, the circulating levels of catecholamines were low and in a few instances the levels were below the HPLC detection limit (i.e. 0.1 nmol l^{-1}). In the eVNP treatment group, the plasma adrenaline concentrations 10 min prior to the NP injection (control), as well as 10 and 30 min post-injection were below the detection limit (Table 1). This prevented the calculation of true means and standard errors at these three sampling times.

In rainbow trout, the long-term and acute cardiovascular effects of bolus injections of NPs (10^{-9} mol kg^{-1}) are shown in Figures 4 and 5, respectively. The long-term effects of NPs were associated with sustained increases in f_H (Figures 4B and 4D). The

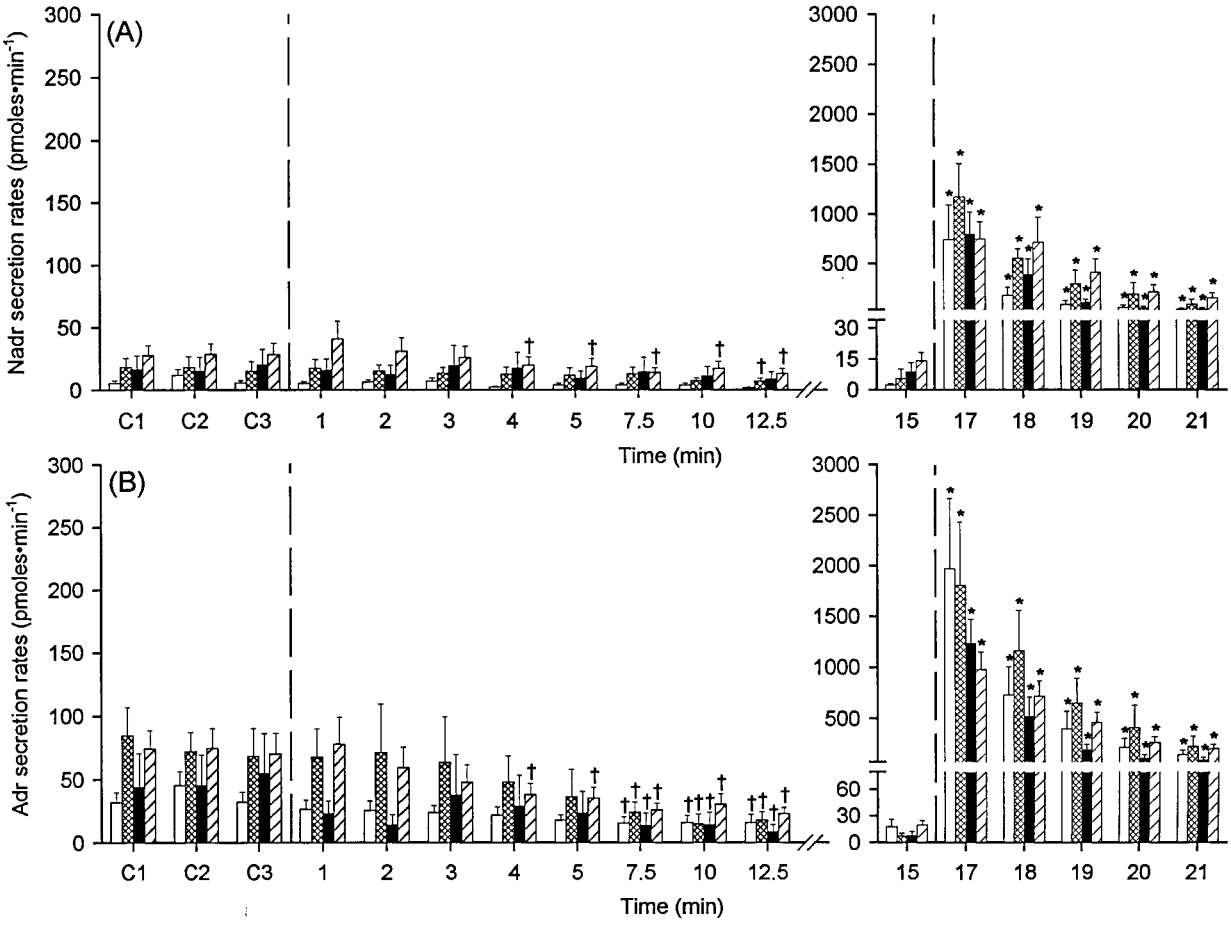

Figure 2. In situ effects of saline (open bars; *N*=9), rat ANP (10^{-9} mol l^{-1}; cross-hatched bars; *N*=6), eel VNP (10^{-9} mol l^{-1}; filled bars; *N*=7), and trout VNP (10^{-9} mol l^{-1}; diagonal-hatched bars; *N*=14) on basal and carbachol-elicited noradrenaline (Nadr; A) and adrenaline (Adr; B) secretion rates in perfused posterior cardinal vein preparations of rainbow trout, *Oncorhynchus mykiss*. The dashed line after C3 (control 3) indicates the switch from saline to the treatments listed above. The dashed line after 15 min indicates when a bolus injection of carbachol (10^{-5} mol kg^{-1}) was given to the preparations. A † symbol and an asterisk denote significant differences from the control C3 and 15 min values for any given treatment, respectively ($p<0.05$). Values are means ± 1 S.E.M.

Table 1. Long-term effects of a 400 μl intra-venous injection of saline, eel ANP (10^{-9} mol kg^{-1}), or eel VNP (10^{-9} mol kg^{-1}) on plasma noradrenaline and adrenaline concentrations in American eel, *Anguilla rostrata*

Time (min)	Plasma noradrenaline (nmol l^{-1})			Plasma adrenaline (nmol l^{-1})		
	Saline (7)	Eel ANP (7)	Eel VNP (7)	Saline (7)	Eel ANP (7)	Eel VNP (7)
−10	0.2±0.1	0.2±0.1	0.5±0.4	0.2±0.1	0.2±0.1	ND
5	0.4±0.2	0.7±0.3	0.7±0.6	0.5±0.3	0.2±0.1	0.5±0.3
10	0.6±0.2	0.5±0.2	0.2±0.1	0.2±0.1	0.5±0.4	ND
30	0.6±0.2	0.8±0.2	0.6±0.5	0.5±0.3	0.8±0.4	ND
60	1.0±0.3	0.9±0.3	0.7±0.6	0.5±0.2	0.6±0.2	0.2±0.1
120	0.8±0.3	0.7±0.4	1.6±1.0	1.0±0.5	0.4±0.2	0.4±0.3
180	0.5±0.2	0.5±0.2	0.4±0.3	0.2±0.1	0.8±0.2	0.7±0.4
360	0.3±0.1	0.5±0.1	0.8±0.5	0.3±0.2	0.5±0.2	0.8±0.4

Values are means ± 1 SEM. *N* values are given in parentheses; ANP, atrial natriuretic peptide; VNP, ventricular natriuretic peptide; ND, non-detectable.

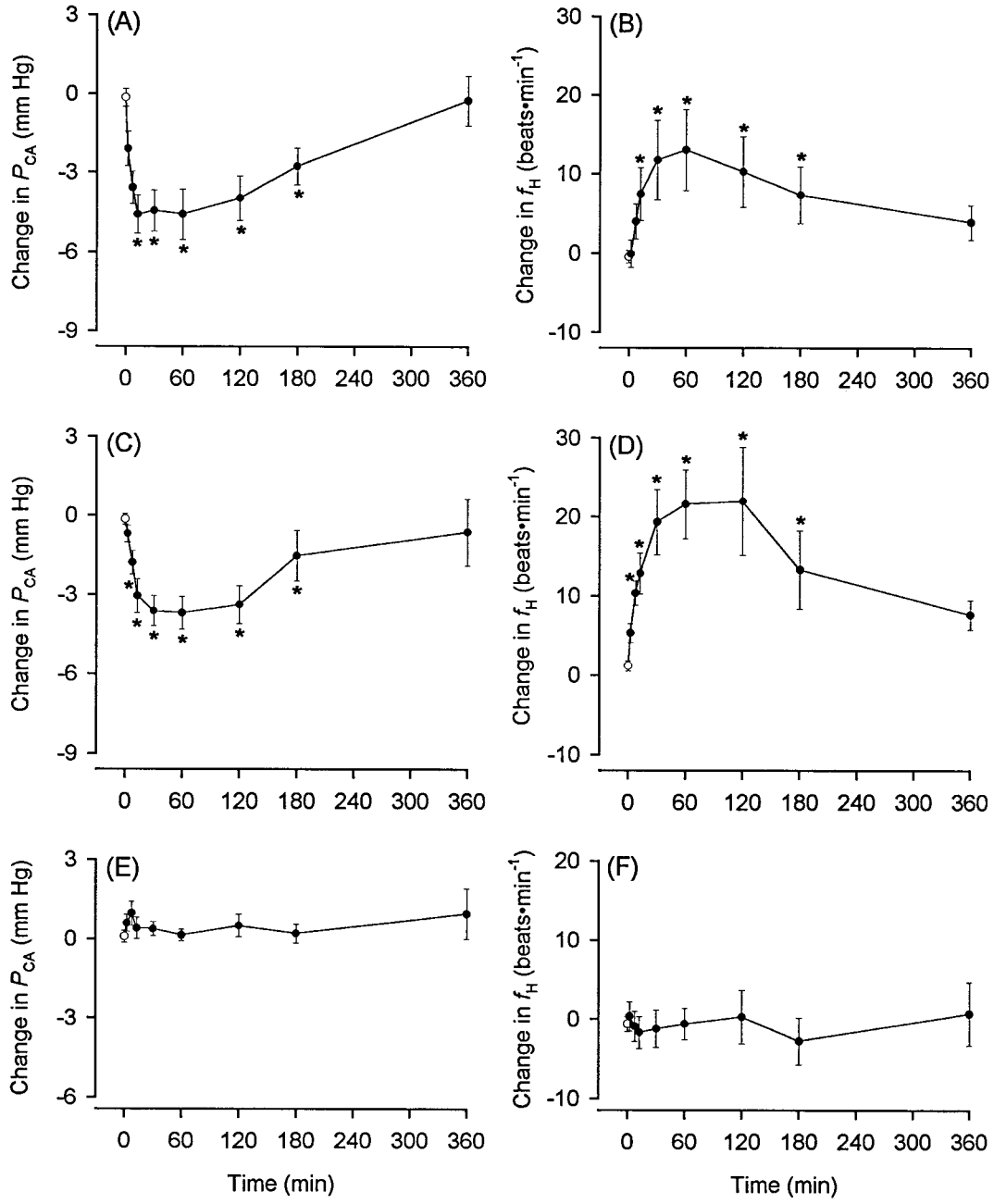

Figure 3. Long-term effects of a 400 μl intra-venous injection of eel ANP (10^{-9} mol kg^{-1}; N=7) (A and B), eel VNP (10^{-9} mol kg^{-1}; N=7) (C and D), or saline (N=13) (E and F) on mean caudal artery pressure (P_{CA}) and heart rate (f_H) in American eel, *Anguilla rostrata*. Within a graph, the open and filled symbols represent the control value (pre-injection) and the response values to a given treatment, respectively. An asterisk denotes a significant difference from the control value for a given treatment (p<0.05). Values are means ± 1 SEM.

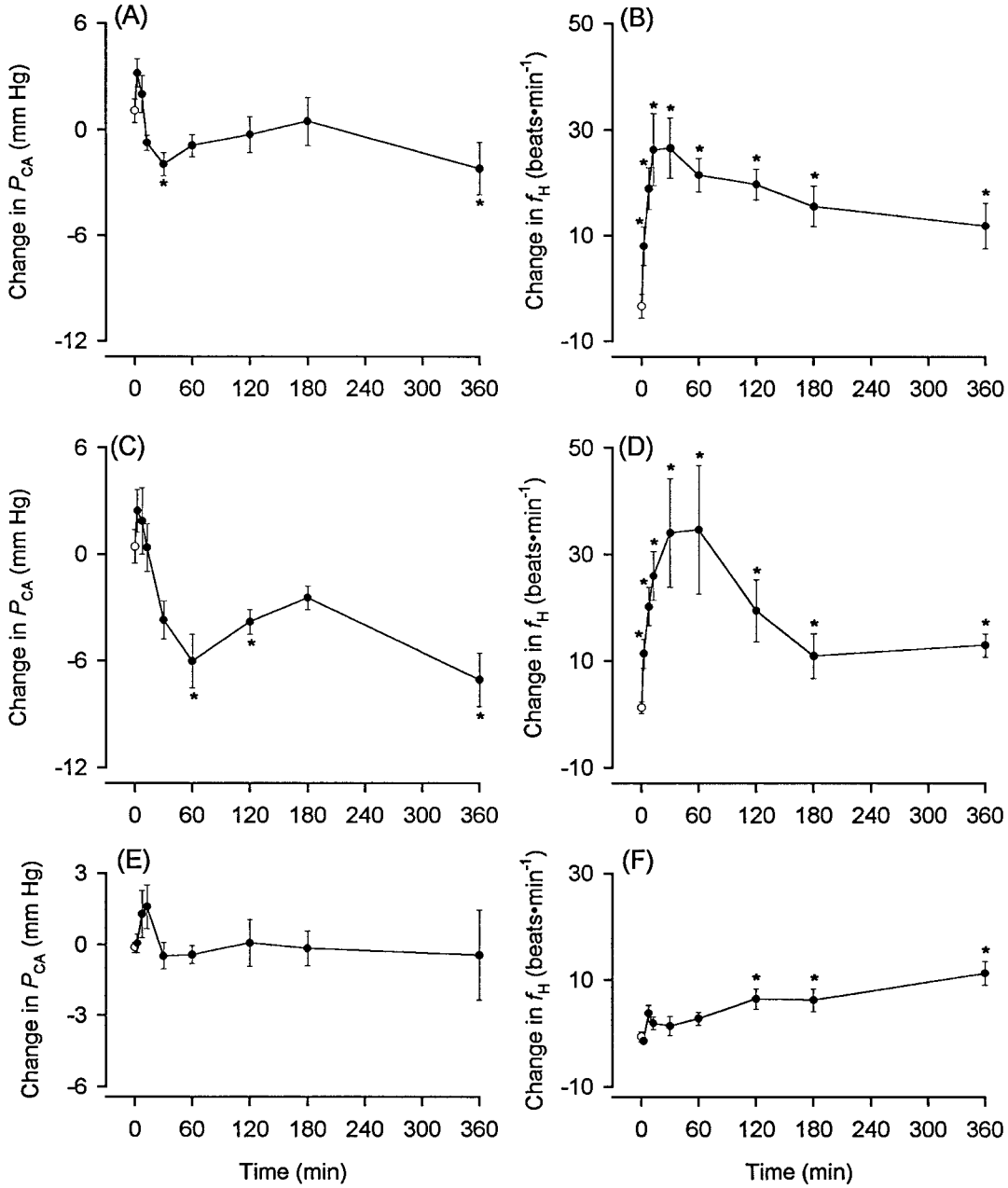

Figure 4. Long-term effects of a 400 μl intra-venous injection of rat ANP (10^{-9} mol kg^{-1}; *N*=6) (A and B), trout VNP (10^{-9} mol kg^{-1}; *N*=6) (C and D), or saline (*N*=7) (E and F) on mean caudal artery pressure (P_{CA}) and heart rate (f_H) in rainbow trout, *Oncorhynchus mykiss*. Within a graph, the open and filled symbols represent the control (pre-injection) value and the response values to a given treatment, respectively. An asterisk denotes a significant difference from the control value for a given treatment ($p<0.05$). Values are means ± 1 SEM.

control pre-injection f_H values were 52.2±3.9 and 51.4±3.3 beats min^{-1} for the rANP and tVNP treatments, respectively. In the control group, saline injection did not affect P_{CA} (Figure 4E). However, there was a significant increase in f_H in the control group that developed after 120 min (Figure 4F). The control pre-injection P_{CA} and f_H values in the saline treatment were 30.6±2.4 cmH_2O and 55.9±4.7 beats min^{-1}, respectively. Both rANP (Figure 5A) and tVNP (Figure 5C) elicited a transient increase in P_{CA}that be-

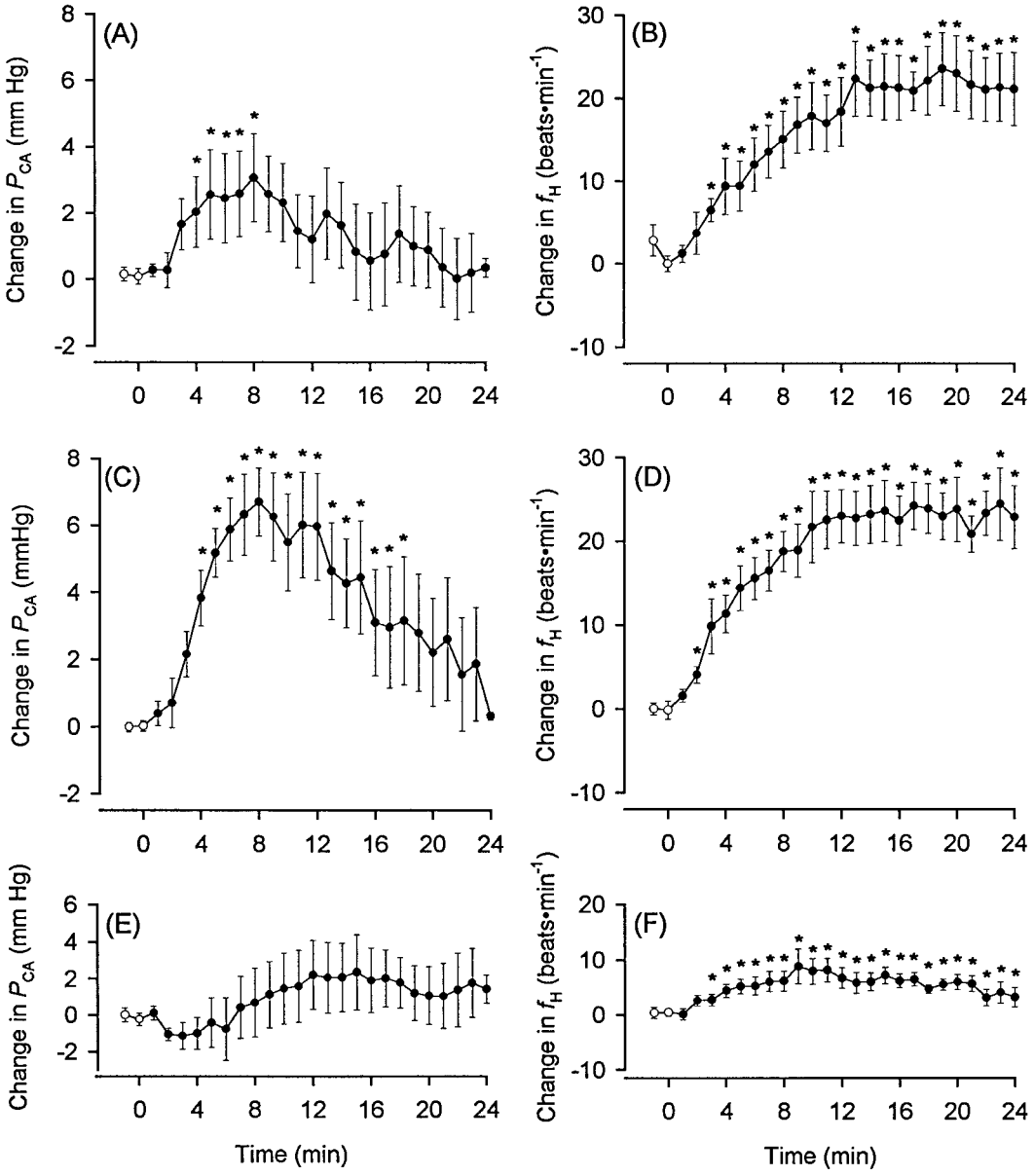

Figure 5. Acute effects of a 400 μl intra-venous injection of rat ANP (10^{-9} mol kg^{-1}; *N*=8) (A and B), trout VNP (10^{-9} mol kg^{-1}; *N*=6) (C and D), or saline (*N*=7) (E and F) on mean caudal artery pressure (P_{CA}) and heart rate (f_H) in rainbow trout, *Oncorhynchus mykiss*. Within a graph, the open and filled symbols represent the control values (pre-injection) and the response values to a given treatment, respectively. An asterisk denotes a significant difference from the control at $t = 0$ value for a given treatment ($p<0.05$). Values are means $\pm$ 1 SEM.

gan 4 min post-injection. The control pre-injection P_{CA} values were 23.5±2.0 and 19.8±1.4 mm Hg for the rANP and tVNP treatments, respectively. The acute hypertensive effects of NPs in the trout were associated with rapid and sustained increases in f_H (Figures 5B and 5D). The control pre-injection f_H values were 53.6±3.5 and 54.8±2.0 beats min^{-1} for the rANP and tVNP treatments, respectively. In the control group, while the saline injection had no significant effect on P_{CA} (Figure 5E) it did elicit a small but significant increase in f_H (Figure 5F); the control pre-injection P_{CA} and f_H values were 22.6±2.5 mm Hg and 54.2±4.5 beats min^{-1}, respectively. After the initial pressor effect of bolus injections of rANP (Figure 5A) or tVNP (Figure 5C) had subsided, P_{CA} fell, and was significantly lower than control val-

ues at 30 and 60 min post-injection, respectively. In both treatments, P_{CA} recovered partially and then fell once more at the 6-h mark (Figures 5A and 5C). The control pre-injection P_{CA} values for the long-term experiments were 30.0±2.4 and 38.9±3.0 cmH_2O for the rANP and tVNP treatments, respectively. Overall, the bolus injections of saline, rANP, or tVNP, either over the long-term (Table 2) or acutely (Table 3), had no significant effect on the plasma concentration of noradrenaline or adrenaline in the trout.

In the spiny dogfish, a bolus injection of dfCNP (10^{-9} mol kg^{-1}) elicited a transient and brief increase in P_{CA} (Figure 6A). This initial pressor effect was followed by a decrease in P_{CA} that was sustained for the duration of the experiment. While R_S also increased within a few min of the dfCNP injection, it gradually returned to the control pre-injection value and remained constant thereafter (Figure 6B). The bolus dfCNP injection also elicited a significant sustained decrease in Q (Figure 6C). The decrease in Q was caused by a reduction in V_S (Figure 6D); f_H remained constant (Figure 6E). The injection of saline in the control fish had no significant effect on any of the measured cardiovascular parameters (Figure 6). dfCNP injections elicited a significant and sustained increase in plasma noradrenaline levels that peaked 10 min post-injection (Figure 7A); plasma adrenaline concentrations were not affected (Figure 7B). In the control group, saline injection alone, had no effect on the circulating concentrations of either catecholamine (Figure 7).

Discussion

The present study demonstrates that homologous and heterologous NPs do not influence basal catecholamine secretion, modulate carbachol-elicited catecholamine release, or change plasma catecholamine levels in two freshwater adapted teleosts, *A. rostrata* and *O. mykiss*. In contrast, however, homologous NP elicit a marked increase in plasma noradrenaline levels in the marine elasmobranch, *S. acanthias*, without affecting plasma adrenaline levels.

The *in situ* perfused PCV preparation has been used previously to investigate the control of catecholamine release in *A. rostrata* (Reid and Perry 1994, 1995) and *O. mykiss* (Fritsche et al. 1993; Reid and Perry 1994; Reid et al. 1994, Reid et al. 1996, Bernier and Perry 1997). The basal and carbachol-elicited catecholamine secretion rates measured in this study are similar to those reported earlier, and as previously shown, are significantly lower in *A. rostrata* than in *O. mykiss* (Reid and Perry 1994). In *O. mykiss*, the time-dependent decrease in basal adrenaline secretion, and to a lesser extent noradrenaline secretion, also have been described previously (Bernier and Perry 1997). Hence, the *in situ* perfused PCV preparations of *A. rostrata* and *O. mykiss* used in this study displayed similar catecholamine release characteristics as those used in previous studies.

The lack of interaction between NPs and basal catecholamine release *in situ* in *A. rostrata* and *O. mykiss* confirm the results of Kloas et al. (1994) obtained using an *in vitro* perifusion preparation of *C. carpio* head kidney slices. Together, these results suggest that NPs do not directly affect basal catecholamine secretion in teleosts. However, while both homologous and heterologous NPs (10^{-9} mol l^{-1}) did not modulate the release of catecholamines elicited by carbachol in this study, perifusion of *C. carpio* head kidney slices with supraphysiological concentrations of heterologous rANP (10^{-7} mol l^{-1}) did elevate ACh-stimulated adrenaline secretion (Kloas et al. 1994). In the same preparation, however, rANP concentrations ranging from 10^{-10} to 10^{-8} mol l^{-1} had no effect on ACh-elicited catecholamine release (Kloas et al. 1994). Given the maximum circulating levels of ANP and VNP recorded in *Anguilla japonica* following acute osmotic or volaemic stress (Kaiya and Takei 1996b), it is unlikely that circulating levels of NPs modulate the cholinergic control of catecholamine release in teleosts. On the other hand, given the ANP-immunoreactivity found in the adrenaline-synthesizing cells of *C. carpio*, it is conceivable that NP concentrations produced locally may modulate adrenaline release in an autocrine or paracrine manner (Kloas et al. 1994).

Although these results are in marked contrast to the well-characterized inhibitory effects of NPs on adrenal medullary catecholamine release in mammals (Fernandez et al. 1992; Vatta et al. 1993, 1994), they concur with observations reported in amphibians (Kloas and Hanke 1992, 1993). In perifusion studies of the adrenal tissues from either the anuran *Xenopus laevis* (Kloas and Hanke 1992) or the urodele *Ambystoma mexicanum* (Kloas and Hanke 1993), rANP had no effect on basal and ACh-elicited catecholamine release. Hence, it would appear that NPs have little, if any, effect on the control of catecholamine release in both teleosts and amphibians.

Table 2. Long-term effects of a 400 μl intra-venous injection of saline, rat ANP (10^{-9} mol kg^{-1}), or trout VNP (10^{-9} mol kg^{-1}) on plasma noradrenaline and adrenaline concentrations in rainbow trout, *Oncorhynchus mykiss*

Time (min)	Plasma noradrenaline (nmol l^{-1})			Plasma adrenaline (nmol l^{-1})		
	Saline (7)	Rat ANP (6)	Trout VNP (6)	Saline (7)	Rat ANP (6)	Trout VNP (6)
−10	1.2±0.4	0.7±0.1	0.9±0.4	0.9±0.3	0.7±0.1	1.0±0.2
5	1.0±0.4	0.8±0.1	0.9±0.5	0.9±0.4	0.6±0.1	1.3±0.3
10	1.1±0.4	0.8±0.2	1.0±0.5	1.1±0.4	0.5±0.1	1.3±0.3
30	1.1±0.4	0.8±0.2	1.3±0.7	1.0±0.3	0.7±0.1	1.2±0.3
60	1.1±0.4	0.8±0.3	1.1±0.5	1.0±0.3	0.7±0.2	1.1±0.3
120	1.0±0.3	0.6±0.1	1.1±0.6	0.8±0.2	0.8±0.3	1.2±0.3
180	1.1±0.3	0.8±0.1	0.9±0.5	0.8±0.2	0.7±0.2	1.1±0.3
360	1.4±0.4	0.9±0.2	0.9±0.4	1.3±0.3	0.8±0.2	1.0±0.2

Values are means ± 1 SEM. *N* values are given in parentheses; ANP, atrial natriuretic peptide; VNP, ventricular natriuretic peptide.

Table 3. Acute effects of a 400 μl intra-venous injection of saline, rat ANP (10^{-9} mol kg^{-1}), or trout VNP (10^{-9} mol kg^{-1}) on plasma noradrenaline and adrenaline concentrations in rainbow trout, *Oncorhynchus mykiss*

Time (min)	Plasma noradrenaline (nmol l^{-1})			Plasma adrenaline (nmol l^{-1})		
	Saline (7)	Rat ANP (8)	Trout VNP (6)	Saline (7)	Rat ANP (8)	Trout VNP (6)
−10	1.1±0.4	0.7±0.1	0.8±0.4	0.5±0.1	0.6±0.1	0.9±0.1
1.5	1.0±0.5	0.8±0.1	0.8±0.4	0.6±0.2	0.6±0.1	1.0±0.2
3	1.0±0.3	0.5±0.1	0.9±0.5	0.9±0.3	0.5±0.1	0.8±0.2
5	1.1±0.4	0.6±0.1	1.0±0.5	0.8±0.1	0.5±0.2	1.3±0.3
10	1.3±0.5	0.6±0.1	1.2±0.5	0.7±0.2	0.7±0.2	1.1±0.4
15	1.0±0.4	0.7±0.1	1.0±0.5	0.8±0.2	0.6±0.2	1.2±0.3

Values are means ± 1 SEM. *N* values are given in parentheses; ANP, atrial natriuretic peptide; VNP, ventricular nutriuretic peptide.

The hypotensive effects elicited by bolus injections of homologous ANP or VNP *in vivo* in *A. rostrata* are characteristic of the cardiovascular effects of NPs previously reported in eels (Takei et al. 1989, 1994b; Oudit and Butler 1995) and confirm the bioactivity of the injections. Confirmation of the bioactivity of the NP's used in the present study was particularly important given the lack of any effects on catecholamine secretion. Clearly, the absence of any effect of NP's on catecholamine secretion *in situ* and *in vivo* in teleosts cannot be explained by insufficient peptide bioactivity. The increases in f_H after NP injections reported here have not been described previously (Oudit and Butler 1995). However, while the eANP dose (5.4×10^{-11} mol kg^{-1}) used by Oudit and Butler (1995) elicited a 15% decrease in dorsal aortic pressure that recovered in 46 min, the eANP dose used in this study (1×10^{-9} mol kg^{-1}) elicited a 24% decrease in P_{CA} which did not recover until 360 min post-injection. Presumably, the increase in f_H was reflexively activated in response to the acute hypotensive effects of the NP injections. Despite the significant cardiovascular changes elicited by eANP and eVNP in *A. rostrata*, the circulating catecholamine levels were unaffected. Thus, the *in vivo* experiments support the results of the *in situ* experiments and strengthen our contention that catecholamine secretion is unaffected by NPs in teleosts. The low, and at times undetectable, circulating catecholamine levels measured in this study are characteristic of eels and have been reported in a number of previous studies (see Randall and Perry 1992 for review).

In *O. mykiss*, bolus injections of either rANP or tVNP produced a characteristic bi-phasic pressor-

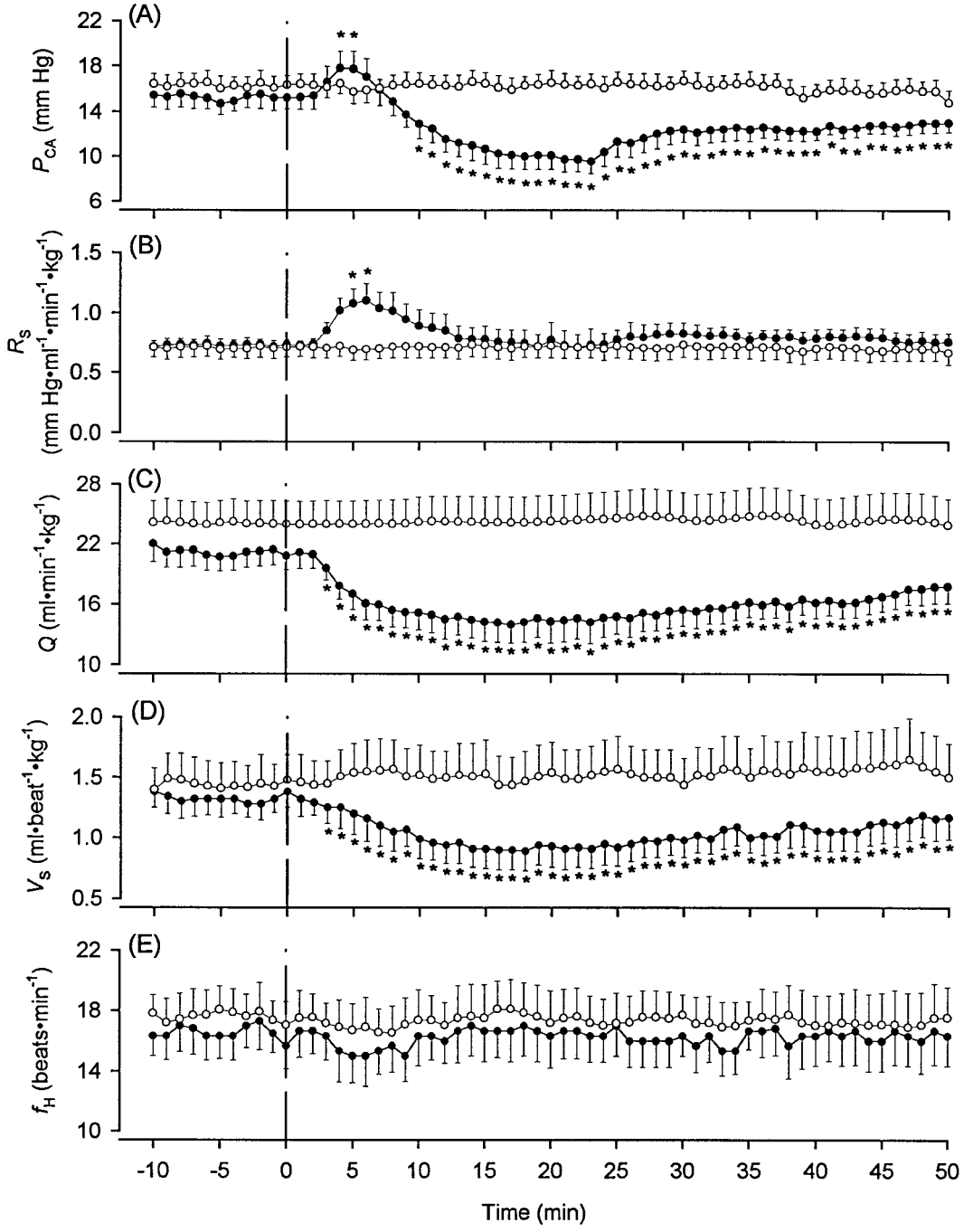

Figure 6. Effects of a 400 μl intra-venous injection of saline (open circles; *N*=6) or dogfish CNP (10^{-9} mol kg^{-1}; filled circles; *N*=6) on mean caudal artery pressure (P_{CA}) (A), systemic vascular resistance (R_S) (B), cardiac output (Q) (C), stroke volume (V_S) (D), and heart rate (f_H) (E) in spiny dogfish, *Squalus acanthias*. The dashed line at time 0 indicates the moment of CNP injection. An asterisk denotes a significant difference from the time 0 control value within a given treatment ($p<0.05$). Values are means $\pm$ 1 SEM.

depressor response (Duff and Olson 1986; Olson and Duff 1992; Takei et al. 1994a; Olson et al. 1997) and an increase in f_H (Olson and Duff 1992; Olson et al. 1997). Although the initial pressor component of this bi-phasic response can be inhibited by α-adrenoceptor antagonists (Olson and Duff 1992; Takei et al. 1994a),

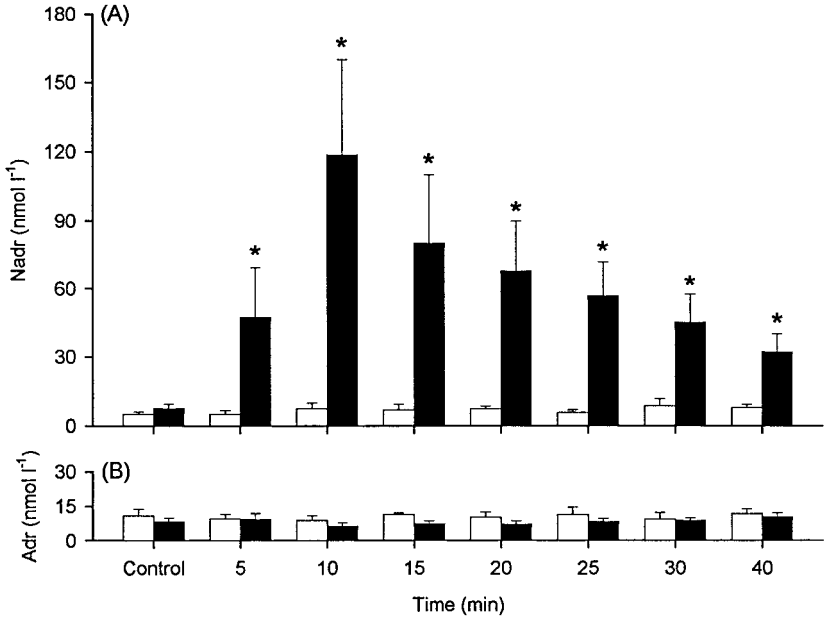

Figure 7. Effects of a 400 μl intra-venous injection of saline (open bars; *N*=6) or dogfish CNP (10^{-9} mol kg^{-1}; filled bars; *N*=6) on plasma noradrenaline (Nadr; A) or adrenaline (Adr; B) concentrations in spiny dogfish, *Squalus acanthias*. An asterisk denotes a significant difference from the saline treatment at a given time ($p<0.05$). Values are means ± 1 SEM.

our results clearly show that it is not mediated by an increase in the levels of circulating catecholamines. Hence, the pressor response to bolus injections of NPs in trout is mediated either via a direct stimulation of sympathetic neurons or, indirectly via a reflexive activation of the sympathetic nervous system (SNS) in response to the cardiovascular actions of NPs (Olson et al. 1997). Because ANP is known to directly inhibit the synthesis and release of noradrenaline from sympathetic terminals in mammals, the ANP-mediated increase in R_S in mammalian species is thought to result from reflex activation of the SNS (Ebert 1988).

Overall, the chronic vasodepressor effects of NPs in *O. mykiss*, as in *A. rostrata*, were not associated with any change in circulating catecholamine levels. Even the temporary recovery in P_{CA} 3 h after the injection of either tVNP or rANP could not be attributed to plasma catecholamines because these remained at basal concentrations throughout the entire 6 h experiment. Instead, the initial and secondary fall in P_{CA} elicited by bolus NP injections may reflect the rapid hypotensive and long-term hypovolemic mechanisms that mediate the cardiovascular effects of NPs in *O. mykiss* (Olson et al. 1997; Duff et al. 1997). Given that NPs have no effect on catcholamine release *in situ*, the possibility that NPs might have inhibited a hypotension-mediated reflex activation of catecholamine release *in vivo* is unlikely. Moreover, in rainbow trout, hemorrhage-induced reductions in blood pressure similar to those elicited by NPs in this study (i.e., 20–25%) do not influence circulating catecholamine levels (N. J. Bernier and S. F. Perry unpublished observations).

In contrast to the *in vivo* results obtained in *A. rostrata* or *O. mykiss*, bolus injections of homologous NPs in the elasmobranch, *S. acanthias*, elicited a marked and sustained increase in circulating noradrenaline levels. Indeed, the circulating noradrenaline levels elicited by homologous CNP in this study are among the highest plasma catecholamine levels recorded in elasmobranchs (Randall and Perry 1992; Perry and Gilmour 1996). Whereas dfCNP injections resulted in a 15-fold increase in plasma noradrenaline levels, circulating adrenaline levels were not altered. Although the dominant catecholamine stored in the axillary bodies of elasmobranchs is noradrenaline, they also contain substantial quantities of adrenaline (see Randall and Perry 1992; Reid et al. 1998, for reviews). Nevertheless, the results of the present study suggest that dfCNP elicited catecholamine release exclusively from noradrenaline-containing chromaffin cells (Reid et al. 1995). Although we cannot determine from this experiment whether dfCNP directly or indirectly stimulated catecholamine release in dog-

fish, the sustained increase in plasma noradrenaline (40 min) elicited by a single bolus injection of dfCNP suggests that part of the effects of CNP may be mediated indirectly. However, since the physiological mechanisms that inactivate NPs and catecholamines have not been investigated in elasmobranchs, and there is a similar lack of knowledge about the dynamics of catecholamine release from the chromaffin cells of the axillary bodies, the nature of the interaction between CNP and catecholamine release in dogfish is inconclusive.

While the long-term vasodepressor effects of dfCNP have been described previously in *S. canicula* (Bjenning et al. 1992), the present study is the first to describe the bi-phasic pressor-depressor response, changes in Q, and R_S associated with dfCNP injections in *S. acanthias*. *In vitro*, dfCNP dilates specific vascular smooth muscle rings of dogfish in a dose-dependant manner (Bjenning et al. 1992; Evans et al. 1993). Although the results from these *in vitro* investigations are only indicative of the potential systemic effects of NPs *in vivo* (Olson and Meisheri 1989), they suggest that the initial pressor effect and increase in R_S associated with dfCNP injections may be mediated indirectly. The CNP-elicited increase in plasma noradrenaline levels may account for the initial increase in systemic vascular resistance. Overall, the cardiovascular changes elicited by CNP in dogfish (i.e. decrease in Q, V_S, P_{CA}, and increase in R_S) are similar to those elicited by rANP or tVNP in *O. mykiss* (Olson et al. 1997). However, whereas NP injections are associated with a long-term increase in f_H in the trout, they have no effect on f_H in the dogfish. This difference may reflect the presence and absence of cardiac adrenergic innervation in trout and dogfish, respectively, and the potential role of circulating catecholamines in cardiac control in these species (Nilsson 1984; Butler and Metcalfe 1988). In *O. mykiss*, the cardiovascular effects of NPs are produced by an initial rapid drop in central venous pressure that results from a NP-stimulated increase in vascular capacitance (Olson et al. 1997). Similarities between the cardiovascular effects of NPs in *S. acanthias* and *O. mykiss* suggest that they may be achieved through a common mode of action. Interestingly, these similarities exist despite a marked difference in the effects of NPs on circulating catecholamine levels. However, while the SNS appears to play a significant role in cardiovascular control in trout, this is not the case in elasmobranchs (Nilsson 1984; Bushnell et al. 1992). Hence, during an acute hypotensive stress, such as that elicited by NPs, elasmobranchs may rely exclusively on circulating catecholamines for adrenergic cardiovascular control (Butler and Metcalfe 1988) whereas trout may elevate systemic vascular resistance through an activation of the SNS.

In summary, while earlier studies suggested that NPs may have a stimulatory effect on the control of catecholamine release in teleosts (Olson and Duff 1992; Kloas et al. 1994), we have found no evidence for such an interaction in either *A. rostrata* or *O. mykiss*. The characteristic depressor effect of NPs in *A. rostrata* and their biphasic pressor/depressor effects in *O. mykiss* do not appear to be associated with a modulation of catecholamine release in these species. In contrast, we have shown that homologous CNP is a potent secretagogue of noradrenaline release in *S. acanthias*, furthermore these circulating catecholamines may contribute to the cardiovascular effects of NPs in elasmobranchs. The mechanism by which CNP elicits catecholamine release in dogfish is unknown and further experiments are required to define the role of NPs in the non-cholinergic control or modulation of catecholamine release in elasmobranchs.

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