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Physiological Inactivation of Vasoactive Hormones in Rainbow Trout

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ABSTRACT Hormone titers are affected by interplay between secretion and inactivation processes. While secretion is a focal process, inactivation mechanisms are often complex and poorly understood. In the present study, inactivation of cardiovascular regulatory hormones was examined from a physiological perspective by analyzing the half-time ($t_{½}$) for recovery of dorsal and ventral aortic and central venous pressure, cardiac output, heart rate, and systemic and branchial vascular resistance following infusion or injection of hormones into conscious rainbow trout, *Oncorhynchus mykiss*. When possible, these were compared to recovery $t_{½}$ of isolated vessel rings in vitro. The $t_{½}$ for epinephrine or norepinephrine recovery in vivo was 3–4 min, approximately twice as long as recovery $t_{½}$ for isolated celiacomesenteric and epibranchial artery rings in vitro. Thus, the rate-limiting step in vascular relaxation is the concentration of circulating catecholamine concentrations, and not metabolic or mechanical events within the vascular wall. The in vivo recovery $t_{½}$ following angiotensin II (ANG II) infusion was 6–7 min, nearly twice that of catecholamines, but also greater than the $t_{½}$ following bolus ANG II injection, inhibition of angiotensin converting enzyme with captopril or injection of trout bradykinin. Arginine vasotocin (AVT) recovery $t_{½}$ in vivo, was considerably longer (20–30 min) than either catecholamine or ANG II $t_{½}$ and longer than AVT recovery $t_{½}$ of isolated vessels in vitro (5–6 min). The inactivation kinetics of catecholamines are consistent with circulatory convection-limited processes and do not appear to be limited by either tissue uptake or enzymatic degradation. This is probably the fastest type of 'on-off' endocrine regulation in fish. Inactivation of ANG II and bradykinin are also convection limited, but ANG II metabolism may become saturated with high doses of exogenous ANG II. AVT inactivation is not convection limited and may compensate for a quantitatively lower capacity of the pituitary for peptide secretion, or less emphasis on AVT as an on/off effector of vascular resistance. *J. Exp. Zool. 279:254–264, 1997.*

Cardiovascular function in fish is affected by a variety of endocrine vasoconstrictory systems including the sympathetic nervous system (SNS; Nilsson, '84; Morris and Nilsson, '94), renin angiotensin system (RAS; Olson, '92), kallikrein kinin system (KKS; Olson, '92; Olson et al,, '97), and arginine vasotocin (AVT; Le Mevel et al., '93; Warne and Balment, '95). Regulation of these systems is dependent upon the ability to elevate hormone titers in response to appropriate stimuli, and the capacity to restore plasma concentrations to resting levels after the stimulus has passed. In all situations, hormone concentrations are affected by interplay between the rates of hormone production (secretion or activation) and inactivation. Secretion/activation processes are better characterized experimentally and they are usually held to be the major factor governing short term regulation of plasma titers. Inactivation processes are technically more difficult to examine, however, recent evidence from mammalian studies suggest that these may also be under physiological control (Davis et al., '92; Kuchel, '94).

Hormone inactivation, from a biochemical perspective, entails removal of the secreted product

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from the circulation either through tissue uptake, excretion, or metabolic transformation. From a physiological perspective, recovery following hormone activation encompasses restoration of the effector to the pre-stimulated state. Physiological recovery includes biochemical inactivation of circulating hormones, dissociation of hormone-effector complexes, and return of intracellular processes to the previous state. If the latter two occur at a slower rate than plasma inactivation, physiological recovery will lag behind plasma recovery; if they are faster, plasma inactivation becomes the rate limiting process. Plasma inactivation can, in turn, be affected by the kinetics of the biochemical processes themselves, or by convective delivery of hormone to the inactivation sites.

The biochemical rate of catecholamine inactivation in fish has been estimated by observing the disappearance of a bolus of unlabeled (Dashow and Epple, '83) or radiolabeled (Ungell and Nilsson, '79, '83; Nekvasil and Olson, '86; Gamperl and Boutilier, '94) catecholamine from the circulation. An approximation of physiological inactivation rate has also been derived from the time course of the fall in arterial pressure after a single bolus catecholamine injection (Wood and Shelton, '80). Most of these studies have shown that the half-time for catecholamines in the circulation is around 2 min which is close to the half-time for convective distribution of inert molecules in the plasma (Gamperl and Boutilier, '94; Nekvasil and Olson, '86).

Comparatively little is known about the inactivation rate of vasoactive peptides. To our knowledge there has been only one study of ANG II inactivation in vivo (Olson et al., '86) and in that, only the disappearance of radio-label following bolus injection of ^{125}I-ANG II was measured. The estimated half-time of ^{125}I in plasma had two rate constants, the first was essentially equivalent to that predicted by convective dilution in the circulation and the second around 12 h (Olson et al., '86). AVT inactivation has not been measured, although Le Mevel ('93) observed that cardiovascular effects of AVT persisted longer than ANG II in trout. Bradykinin turnover has not been measured in fish.

There are several problems inherent with most studies of inactivation rate of vasoactive hormones in fish. First, when plasma hormone concentrations are monitored following a single intravascular bolus, it is difficult to separate biochemical inactivation from convective and diffusive dilution of the hormone in blood and interstitium. Second, when inactivation is determined from the clearance of radioactivity following injection of a labeled hormone, the rate of clearance of radioactivity does not correct for production of radio-labeled metabolites and their subsequent addition to, or clearance from, the circulation. Third, when a physiological variable, such as recovery of blood pressure, is used as an index of hormone inactivation, the actual rate of hormone clearance from the plasma may be masked by dilution of the injected bolus, biochemical, or mechanical processes associated with smooth muscle and cardiac recovery, or nonsynchronous changes in resistance upstream and downstream from the measurement site. However, the time course of pressure restoration probably provides a more physiologically relevant picture of the recovery process than measuring the rate of physical removal of the stimulant from the circulation, if the above errors in this method can either be controlled or compensated.

The purpose of the present experiments was to examine inactivation of cardiovascular hormones in trout from a physiological perspective by measuring the recovery rate of cardiovascular variables in vivo following hormone infusion or injection. These data were compared to the recovery rate of isolated vessels in order to distinguish local from general plasma inactivation kinetics.

MATERIALS AND METHODS

Animals

Rainbow trout (*Oncorhynchus mykiss*; 400–750 g), of either sex, were obtained from a local hatchery and kept in circulating 2,000-liter tanks at 12°C and under appropriate, seasonal light:dark cycles. Fish were fed a maintenance diet of commercial trout pellets (Van Den Bosch, Zeeland, MI) up to 48 h prior to experimentation.

In vivo infusion protocol

Details of trout cannulation are described in Olson et al. ('97). Trout were anesthetized in benzocaine (ethyl-*p*-aminobenzoate; 1:12,000, W:V) and placed ventral side up in a "V" trough. The dorsal aorta was cannulated percutaneously via the roof of the buccal cavity with PE 60 and the cannula exteriorized dorsally through the snout. This procedure took less than 1 min and the gills were not irrigated. Thereafter, gills were continuously irrigated with cold (5–10°C) aerated water containing 1:24,000, W:V, benzocaine. The heart was exposed with a midline ventral incision and the ductus Cuvier was cannulated with 0.51 mm-I.D. Silastic tubing (Dow Corning medical grade, PGC Scientifics, Gaithersburg, MD). A clamp was

then placed between the ventricle and the bulbus and a silastic cannula was inserted into the bulbus near the bulboventricular junction. After the clamp was removed, a 3S Transonic flow probe (Transonic Systems Inc., Ithaca, NY) was placed around the ventral aorta distal to the site of the cannula insertion and connected to a Transonic T101 flow meter. The wound was closed with interrupted silk sutures and sealed with cyanoacrylate gel (super glue gel). Ventral cannulas and flow probe cable were secured to the abdomen, pelvic and caudal fins with silk sutures. All cannulas were filled with heparinized saline (100 USP units · ml^{-1} heparin in 9.0 g/l NaCl) and connected to pressure transducers. The fish were revived and placed in black plastic tubes in a 1,000 l tank with through-flowing aerated well water at 12°C. Experiments were conducted 48 h after surgery. An in-line four-way stopcock in the dorsal aortic cannula served as the site for hormone infusion.

Resting pressure and cardiac output were visually monitored for 1–2 h prior to experimentation to insure that they were stable and within normal values for resting trout. Control values were continuously collected by computer for 5 min prior to hormone infusion and throughout the remainder of the experiment. Epinephrine or norepinephrine (1×10^{-4} mol·l^{-1}) or ANG II (1×10^{-5} mol·l^{-1}) were infused with a syringe infusion pump (model 22, Harvard Apparatus, South Natick, MA) at the rate of 0.3 mol·min^{-1} for 1 min to flush the cannula and to prime the fish. The fish was then continuously infused at 1–2 ml·hr^{-1} for 20–30 min, to achieve steady-state cardiovascular parameters for at least 15 min. The actual infusion rate for each fish was adjusted to elevate dorsal aortic pressure by 5–10 mmHg. The stopcock was then closed to the pump and cardiovascular parameters were recorded for an additional 30–45 min until they returned to preinfusion levels.

Analog pressure signals were recorded with Hewlett Packard 7853A patient monitors (Palo Alto, CA). Digitized signals of pressure and flow were collected every 0.1 sec and 1 sec averages were stored in a computer. The pressure transducers were calibrated with a water manometer and the flowmeter was calibrated in situ at the end of the experiment by pump perfusion of the ventricle with 12°C saline at known flow rates. Heart rate was derived by the computer from either the ventral aorta or systolic pulse interval. Systemic vascular resistance (Rs) was calculated by dividing dorsal aortic pressure (pDA) minus ductus Cuvier pressure (pDC) by cardiac output (CO):

$$Rs = [pDA–pDC] \cdot CO^{-1} \quad (1)$$

Gill vascular resistance (Rg) was calculated as the difference between ventral aortic (pVA) and dorsal aortic pressures divided by cardiac output:

$$Rg = [pVA–pDA] \cdot CO^{-1} \quad (2)$$

Both resistances were calculated from the one-second averaged pressure and flow values.

Typical control values were: pDA, 24.1 ± 1.5 mmHg; pVA, 34.8 ± 2.5 mmHg; pVEN, 2.6 ± 0.5 mmHg; CO, 20.6 ± 2.7 mm·min^{-1}·kg^{-1}; HR, 59.4 ± 3.2 beats·min^{-1}; Rs, 1.2 ± 0.2 mmHg·ml^{-1}·min^{-1}·kg^{-1}; Rg, 0.5 ± 0.1 mmHg·ml^{-1}·min^{-1}·kg^{-1}. Saline infusion did not affect any of these parameters.

In vivo bolus injection protocol

The time course of the effects of a single bolus injection of hormone, or of the angiotensin converting enzyme (ACE) inhibitors, captopril or lisinopril, on cardiovascular parameters was examined in trout instrumented as above, or in trout fitted with only a dorsal aortic cannula. Injected doses were: Epi (1×10^{-9} mol·kg^{-1}), ANG II ($1 \times$ mol·kg^{-1}), captopril or lisinopril (3×10^{-8} mol·kg^{-1}), and AVT (1×10^{-10} mol·kg^{-1}). ANG II was also injected after captopril pre-treatment. Trout bradykinin ([Arg^0, Trp^5, Leu^8]bradykinin; tBK; 1×10^{-8} mol·kg^{-1}) was injected into trout pretreated with 0.5 mg·kg^{-1} indomethacin as part of a separate study (Olson et al., '97). All hormones were given in a single 0.25–0.5 ml bolus into the dorsal aorta cannula followed by an additional 0.3 ml saline flush. Data was collected as above for 5 min prior to, and 30 (Epi, ANG II and captopril) or 60 (AVT, tBK) min thereafter. Bolus saline injection did not affect pDA.

Isolated vessels

Trout were stunned by a blow to the head and the spine was severed. Celiacomesenteric (CM) and third- or fourth-arch efferent branchial (EB) arteries were removed and placed in 4°C phosphate-buffered saline (PBS). Loose connective tissue and blood was removed from the adventitia and a pair of 1 to 2 mm-wide rings were cut from the center of each vessel. The endothelium was not removed in these experiments. Two 280 μm diameter steel "S" hooks were used to fasten the rings between a Grass FT03C force-displacement transducer and support rod and the rings were suspended in individual 20 ml water-jacketed (12 ± 0.2°C) smooth muscle chambers and aerated with room air.

The rings were equilibrated for 1 h at 0.75 (CM)

or 0.5 (EB) grams tension to establish a constant resting tension. They were then contracted with 1×10^{-5} mol·l^{-1} epinephrine for 5 min and washed with four rinses of PBS over the next hour and resting tension was reestablished. In the catecholamine studies, one ring from each pair was then pretreated (10–15 min) with the beta antagonist, propranolol (1×10^{-5} mol·l^{-1}) to prevent any β-mediated relaxation concurrent with the contraction (Olson and Meisheri, '89). The rings were then contracted with 1×10^{-7} mol·l^{-1} epinephrine or norepinephrine or 1×10^{-9} mol·l^{-1} arginine vasotocin (AVT) until tension plateaued (~10 min). The chambers were then flushed with 25 ml PBS, drained, and the vessels quickly washed with a gentle stream of 10°C PBS. The chambers were flushed a second time, filled, and the vessels allowed to relax. In other experiments, the vessels were pretreated with the monoamine oxidase inhibitor, pargyline (10^{-5} mol·l^{-1}) prior to epinephrine treatment. Neither ANG II nor tBK contract large systemic arteries in trout (Olson et al., '94; Conlon et al., '96) and they were not examined in this study.

Relaxation kinetics following a ligand-independent contraction were examined in a separate group of experiments by depolarizing vascular smooth muscle with high potassium. Rings of EB were contracted by addition of potassium chloride to the chamber (final concentration 50 mM K^+) and relaxation was initiated by replacing the bath with PBS after tension had stabilized for ~10 min.

Vessel tension was recorded with Gould chart recorders (model 8188, Gould Instruments, Cleveland, OH). The chart recordings were optically scanned into the computer and digitized for curve-fit analysis.

Curve analysis

Rate constants for the decline in cardiovascular parameters at the end of hormone infusion, or the drop in artery tension in vitro, were determined with curve-fitting software (Jandel Scientific Software, San Rafael, CA). It was assumed that these curves followed a single or two-component exponential decay with one or two rate constants and could be defined by the equations:

$$A_t = A_{eq} + A_{1t}e^{-k_1 t} \quad (3)$$

$$A_t = A_{eq} + A_{1t}e^{-k_1 t} + A_{2t}e^{-k_2 t} \quad (4)$$

Where: A_t is the activity (pressure, resistance, tension, etc.) at any time, t. A_{eq} is activity at equilibrium (rest). A_{1t} and A_{2t} are activities due to components 1 and 2, respectively and $-k_1$ and $-k_2$ are the rate constants for the fast and slow components, respectively. The half-time ($t_{½}$) is calculated from the rate constant: $(t_{½}) = 0.693 \cdot k^{-1}$. Only the fast component was examined in detail in the present study. In practice, it was evident that when two components were present the half-time f the second (slow) component was one to two orders of magnitude longer than the first and probably was of little overall significance in accounting for most of the observed decline in physiological activity. Additionally, the presence of a second component had little affect on the value of the rate constant of the first component.

Values were compared by one-way ANOVA or Mann-Whitney Rank Sum test and expressed as means ± SE.

Chemicals

Catecholamines, salmonid [Asn^1, Val^5] angiotensin II, arginine vasotocin, indomethacin, pargaline, and captopril were purchased from Sigma (St. Louis, MO). tBK, (Arg-Arg-Pro-Pro-Gly-Trp-Ser-Pro-Leu-Arg; Conlon et al., '96) was synthesized by Chiron Mimetopes Peptide Systems (San Diego, CA), and purified by reverse phase HPLC on a Vydac 218TP54 column. Lisinopril was a generous gift from Merck, Sharp and Dohme Research Laboratories (Rahway, NJ). All other chemicals were reagent grade. Phosphate buffered saline (PBS) consisted of (in g·l^{-1}) 7.37 NaCl, 0.31 KCl, 0.10 $CaCl_2$, 0.14 $MgSO_4$, 0.46 KH_2PO_4, 2.02 Na_2HPO_4; pH adjusted to 7.8. Vessel rings were incubated in PBS containing 0.9 g·l^{-1} glucose.

RESULTS

The general protocols for hormone infusion or bolus injection are shown in the case of norepinephrine (Nepi) in Figures 1 and 2, respectively. After an initial 5 min control period, Nepi was infused at a rate of 6×10^{-8} mol·kg^{-1}·min^{-1} for 1 min (prime) and then at 5×10^{-9} mol·kg^{-1}·min^{-1} for an additional min. During the initial 5–10 min of the infusion period pressures and flow were quite variable, becoming stable thereafter. Nepi infusion was stopped after 20 min (26 min elapsed from start of record) and the cardiovascular variables returned toward baseline at an exponential rate. Figure 2 shows response and subsequent decline in cardiovascular parameters after a bolus Nepi injection. A typical curve fit analysis for the drop in ventral aortic pressure from Nepi infusion (data from Fig. 1) is shown in Figure 3. This

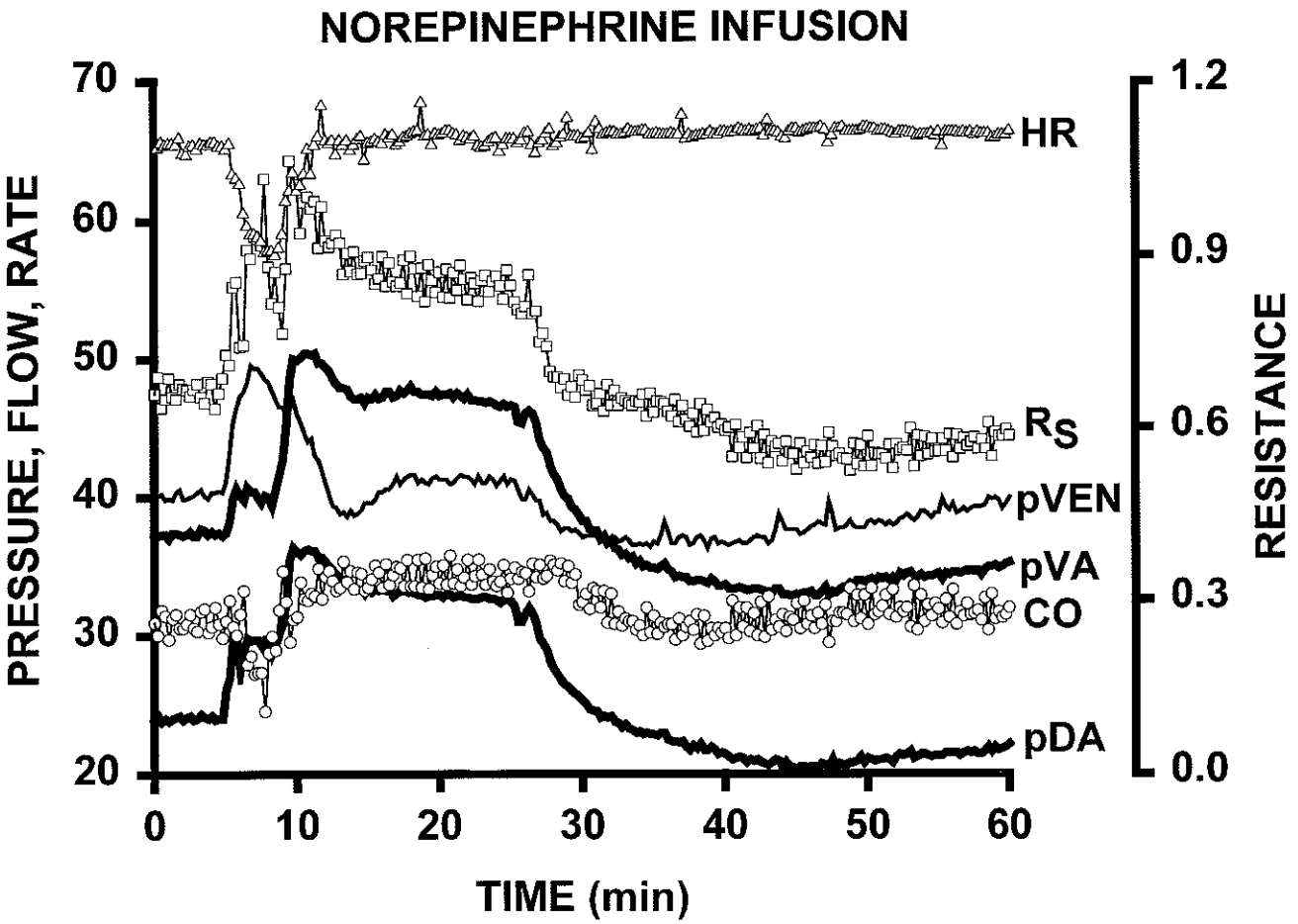

Fig. 1. General protocol for hormone infusion in conscious trout. At the end of a 5 min control period norepinephrine was infused at the rate of 6×10^{-8} mol·kg^{-1}·min^{-1} for 1 min (prime) and then at 5×10^{-9} mol·kg^{-1}·min^{-1} for an 20 additional min. Dorsal (pDA, heavy lower line) and ventral (pVA, heavy upper line) aortic and ductus Cuvier (pVEN, thin line; ×10) pressures, cardiac output (CO, ○), heart rate (HR, △) and calculated systemic resistance (R_S, □) fluctuated during the first 10 min of infusion and became stable thereafter. When infusion was terminated at 26 min these parameters recovered at an exponential rate.

follows a mono-exponential decline with a rate constant of 0.24 min^{-1} and a 2.9 min $t_{½}$ (Fig. 3). Relaxation of the celiacomesenteric artery in vitro after Nepi exposure was also mono-exponential with a 0.9 min^{-1} rate constant and 0.8 min $t_{½}$ (Fig. 4).

Relaxation $t_{½}$ for infused or injected hormones/inhibitors are shown in Tables 1 and 2, respectively. Table 3 shows relaxation $t_{½}$ for isolated vessels. In vivo $t_{½}$ for epinephrine (Epi) or Nepi infusion, Epi injection, angiotensin converting enzyme (ACE) inhibition, or angiotensin II (ANG II) injection (with or without ACE inhibition), were not significantly different. Half-times for ANG II infusion, with or without ACE inhibition, were similar, and they were approximately twice as long as the catecholamine $t_{½}$ ($P \leq 0.05$). Catecholamine relaxation $t_{½}$ in vivo was twice as long as that for celiacomesenteric arteries in vitro. AVT injection $t_{½}$ was nearly an order of magnitude longer than that for catecholamines ($P \leq 0.05$). Arteries exposed to AVT in vitro took twice as long to relax compared to arteries exposed to catecholamines ($P \leq 0.05$), whereas AVT relaxation in vivo was significantly ($P \leq 0.05$) slower than AVT relaxation in vitro. Relaxation $t_{½}$ of EB following 50 mM K^+ contraction was not significantly different from catecholamine contracted vessels, but was significantly ($P \leq 0.05$) slower than pDA and pVA pressure recovery in vivo. The $t_{½}$ after bolus injection of trout bradykinin (tBK) was similar to the $t_{½}$ for catecholamines, ACE inhibitors, and ANG II injection. The monoamine oxidase inhibitor, pargaline, did not affect the rate of Epi relaxation of celiacomesenteric arteries. Other than AVT, most treatments did not produce consistent response in either cardiac output or gill resistance. The

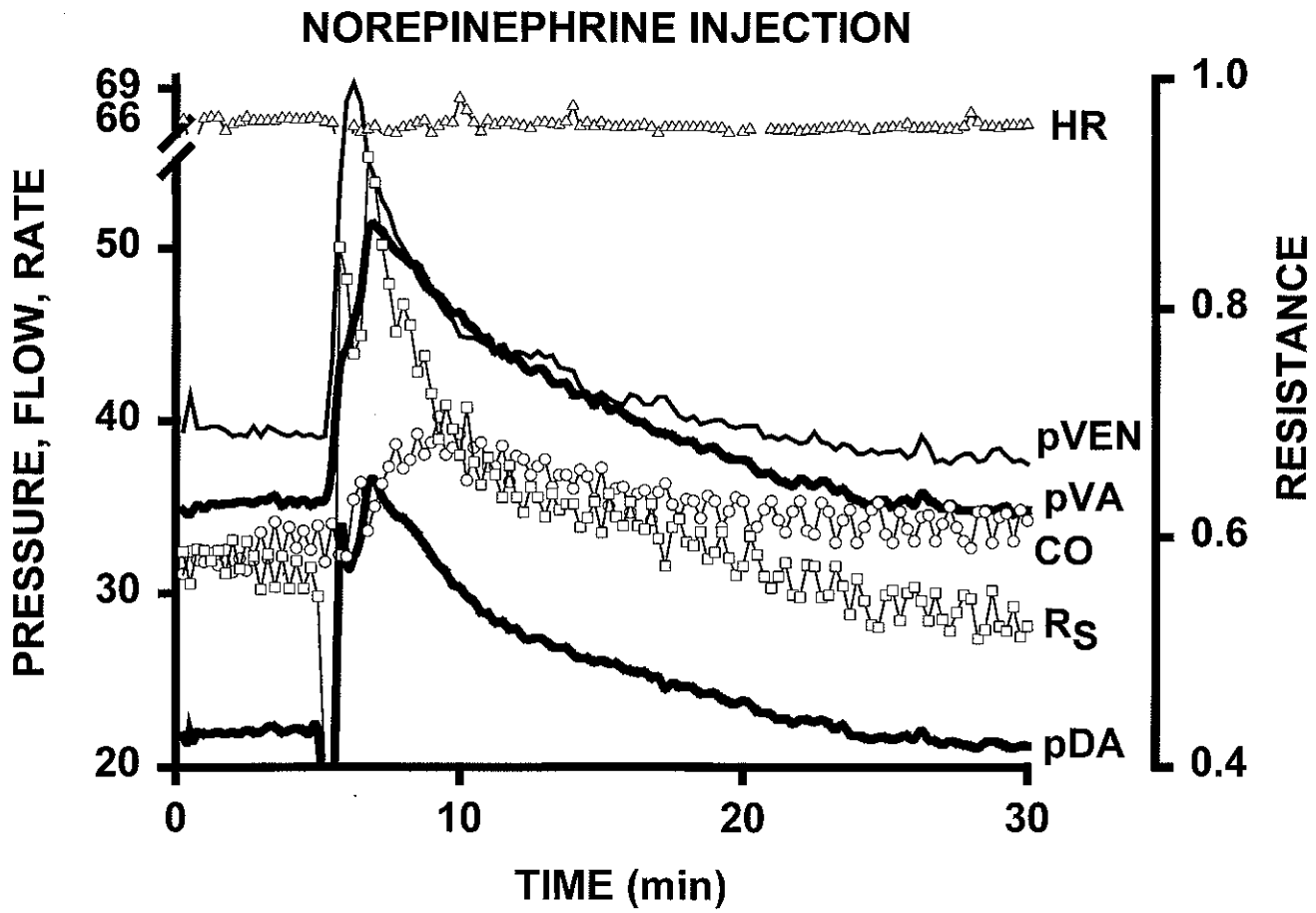

Fig. 2. General protocol for bolus hormone injection in conscious trout. At the end of a 5 min control period a single, 1×10^{-6} mol·kg^{-1}, bolus of norepinephrine was injected and cardiovascular variables were monitored for 25 min. Symbols as in Figure 1.

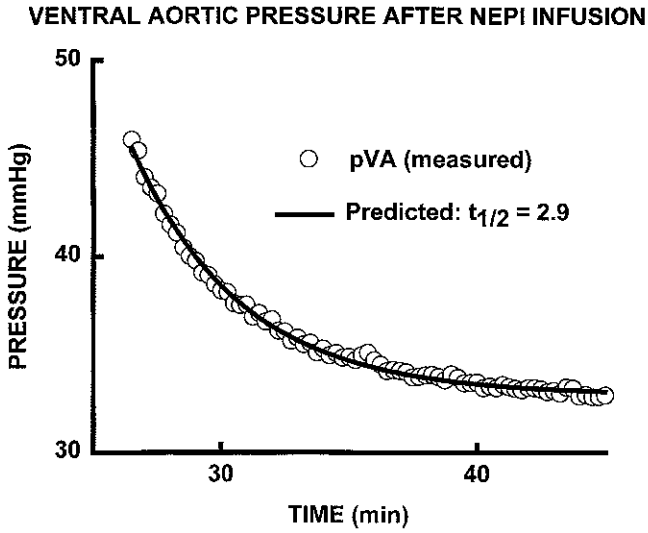

Fig. 3. Curve-fit analysis of the fall in ventral aortic pressure after norepinephrine infusion. Open circles (○) are data from Figure 1, solid line is the predicted mono-exponential decay with a half-time of 2.9 min.

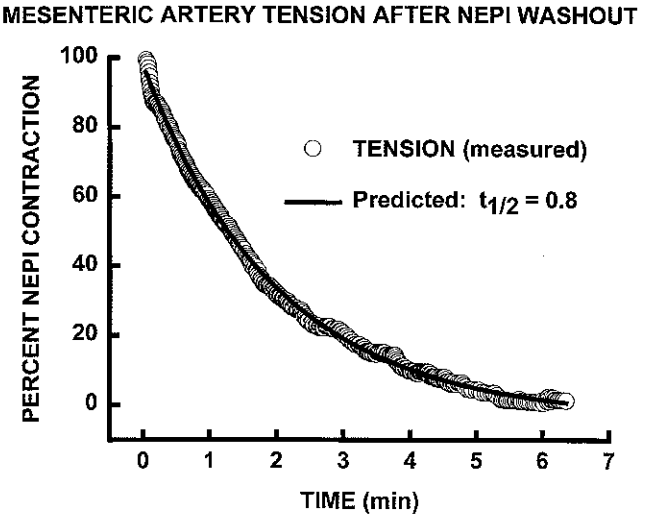

Fig. 4. Curve-fit analysis of the relaxation of an isolated celiacomesenteric artery after removal of 10^{-7} mol·l^{-1} norepinephrine from the bathing medium. Open circles (○) indicate measured artery tension as a percent of maximum contraction, solid line is the predicted mono-exponential decay with a half-time of 0.8 min.

TABLE 1. Half-time (in min) for relaxation of cardiovascular variables in vivo following continuous infusion[1]

	Epi	Nepi	ANG II	Capto + ANG II
pDA	2.8 ± 0.2 (21)	3.9 ± 0.5 (8)	5.2 ± 0.5[2] (9)	6.8 ± 0.8[2] (13)
pVA	3.4 ± 0.3 (21)	4.3 ± 0.7 (8)	6.1 ± 1.0[2] (9)	
pVEN	3.2 ± 0.8 (21)		7.0 ± 1.3[2] (8)	
Rs	3.1 ± 0.5 (21)	3.7 ± 0.6 (6)	6.1 ± 1.3[2] (7)	

[1]Epi, epinephrine; Nepi, norepinephrine; ANG II, angiotensin II; Capto, captopril; pDA, dorsal aortic pressure; pVA, ventral aortic pressure; pVEN, central venous pressure; Rs, systemic resistance; blank space indicates parameter not mesured; n.c., no consistent change observed. Means ± SE.
[2]All ANG II half-times are significantly greater than corresponding Epi half-times ($P \leq 0.05$).

predominant cardiovascular response to AVT injection was an increase in gill resistance.

DISCUSSION

Ungell and Nilsson ('79, '83) provided the first estimate for the rate of vasoactive hormone clearance from fish plasma by measuring the rate of disappearance of radioactive epinephrine (^{3}H-Epi) from dogfish (*Squalus acanthias*) and cod (*Gadus morhua*) plasma following bolus injection. They found that clearance had two components. Analysis of their data (Ungell and Nilsson, '79, '83) using the curve-fit method of the present study shows that the fast component has a 5.4 min $t_{½}$ in *S. acanthias* and 1.9 min $t_{½}$ in *G. morhua* (Table 4). Since then, a number of investigators have shown that plasma catecholamines fall rapidly after bolus injection (Table 4).

It is evident from studies on trout, *Oncorhynchus mykiss* (Nekvasil and Olson, '86; Gamperl and Boutilier, '94), that passive dilution of label from the injection site could account for much, if not all, of the fast component because the $t_{½}$ for the decrease in plasma ^{3}H-catecholamine and ^{14}C-sucrose (an inert extracellular volume marker) following bolus injection are very similar (Table 4). If catecholamine clearance is corrected for passive dilution by normalizing ^{3}H-catecholamine clearance to ^{14}C-sucrose clearance (Nekvasil and Olson, '86; Gamperl and Boutilier, '94) and examined by curve fitting, the corrected fast component $t_{½}$ for Epi is 1.7 (calculated from Nekvasil and Olson, '86) or 2.4 (calculated from Gamperl and Boutilier, '94) min and for Nepi is 0.8 min (calculated from Nekvasil and Olson, '86). Because these $t_{½}$ fall within the $t_{½}$ of passive dilution of ^{14}C-sucrose, there is considerable uncertainty regarding their accuracy. In the present study, the problem of passive dilution was circumvented by infusing unlabeled catecholamines or angiotensin II (ANG II) until measurable, but not unphysiological, changes in cardiovascular parameters were achieved and maintained under steady-state conditions. It is presumed that when the cardiovascular parameters are at their new steady state, an elevated, steady-state plasma catecholamine or ANG II concentration is also attained. Because the blood recirculation time in trout is one minute or less (Olson and Duff, '93), a 20 min infusion period will permit at least 20 circulation times for mixing.

When catecholamine infusion was stopped, all cardiovascular variables that were affected by elevated plasma catecholamines decayed at approximately the same rate with a $t_{½}$ of around 3 min (Table 1). The $t_{½}$ for physiological recovery from

TABLE 2. Half-time (in min) for relaxation of cardiovascular variables in vivo following bolus injection[1]

	Epi	ANG II	Captopril	ANG II (+ capto)	Lisinopril	Lisinopril	AVT	tBK
pDA	3.3 ± 0.5 (11)	3.8 ± 0.1 (3)	3.2 ± 0.3 (7)	3.5 ± 0.3 (13)	4.2 ± 0.4 (12)	4.0 ± 0.3 (37)	18.0 ± 6.6[2] (4)	3.0 ± 0.3 (4)
pVA	4.0 ± 0.6 (11)	4.0 ± 0.6 (3)			3.7 ± 0.6 (11)		30.8 ± 2.1[2] (5)	4.3 ± 1.1 (4)
pVEN	4.4 ± 1.4 (5)	4.0 ± 0.5 (3)			4.5 ± 0.9 (10)			3.3 ± 0.7 (4)
CO	6.0 ± 1.6 (8)							
Rs or Rg*	2.1 ± 0.6 (7)				5.0 ± 0.9 (8)		30.0 ± 9.2[2*] (4)	2.2 ± 0.4 (4)

[1]Epi, epinephrine; ANG II, angiotensin II; Capto, captopril; AVT, arginine vasotocin; tBK, trout bradykinin pDA, dorsal aortic pressure; pVA, ventral aortic pressure; pVEN, central venous pressure; CO, cardiac output; Rs, systemic resistance; Rg, gill resistance, blank space indicates parameter not measured or no consistent change observed. Means ± SE.
[2]All AVT values significantly greater than all corresponding values for other hormones ($P \leq 0.05$).

TABLE 3. Half-time (in min) for relaxation of celiacomesenteric (CM) or efferent branchial (EB) artery rings in vitro following continuous exposure[1]

	Epi	Epi + Par	Nepi	AVT	50 mM K^+
CM	1.6 ± 0.3 (12)	1.3 ± 0.1 (9)	1.7 ± 0.4 (5)	4.9 ± 0.3 (4)	
EB	1.4 ± 0.6 (3)			6.5 ± 0.7 (8)	2.1 ± 0.2 (15)

[1]Epi, epinephrine; Par, pargaline; Nepi, norepinephrine; AVT, arginine vasotocin; 50 mM K^+, 50 mM potassium chloride. Means ± SE. All catecholamine values significantly less than corresponding in vivo parameters (Tables 1 and 2) for identical hormone ($P \leq 0.05$). All AVT values signficantly greater than catecholamine values or 50 mM K^+ ($P \leq 0.05$). All 50 mM K^+ values significantly less than corresponding in vivo parameters (Tables 1 and 2) except pVEN, Rg, and Rs ($P \leq 0.05$) but not different from in vitro catecholamine values in this table.

catecholamine infusion (Table 1) is also similar to the half-time for physiological recovery from a catecholamine bolus (Table 2) and to the half-time for passive dilution of ^{14}C-sucrose observed by Gamperl and Boutilier ('94; also see Table 4). Because isolated vessels relax twice as fast in vitro (Table 3) as they do in vivo (table 1), it is doubtful if biochemical or mechanical events in the vessel wall are rate limiting factors in blood pressure recovery. Also, because isolated vessels relax at the same rate after the receptor-mediated catecholamine contraction as they do after a voltage-mediated 50 mM K^+ depolarization, ligand-receptor interactions are not rate limiting. These observations indicate that convection of catecholamines through the vasculature, but not tissue uptake, enzymatic degradation, or receptor dissociation, is the rate limiting step in catecholamine inactivation. The observation by Gamperl and Boutilier ('94) that the rate of ^{3}H-epinephrine disappearance from trout plasma is unaffected by simultaneous injection of unlabeled epinephrine is consistent with a convective rather than either a cellular uptake or enzymatically limited process.

The cardiovascular effects of ANG II infusion decayed with a 6 min $t_{½}$, around twice as long as that needed for convective mixing. Because ANG II does not constrict large vessels (Olson et al., '94) it was not possible to determine if the prolonged ANG II relaxation was due to a decreased rate of ANG II dissociation from vasculature receptors or to slower plasma clearance. Perhaps of some relevance is the fact that the in vitro $t_{½}$ of another constrictor peptide, AVT, is three times longer than that of the catecholamines (Table 3; although see below).

The $t_{½}$ for pDA recovery from ANG II infusion is longer than that following an ANG II bolus ($P \leq 0.05$) or the development of hypotension after injection of the angiotensin converting enzyme (ACE) inhibitor, captopril ($P \leq 0.05$; Tables 1, 2). The time course for both ANG II injection and ACE inhibition (~3 min) are more consistent with convection limited processes. Because the total amount of ANG II injected was considerably less (< 1%) than the amount of ANG II infused, prolonged infusion may have overloaded the catabolic capacity and resulted in a decreased recovery rate.

It is less clear why the onset of hypotension following ACE inhibition was also rapid. The short $t_{½}$ for ACE inhibition is consistent with a convection-limited process. This implies that the rate-limiting step is the convective delivery of inhibitor to the enzyme and that neither the rate of inhibitor-enzyme association nor the rate of inactivation of circulating ANG II formed prior to ACE inhibition are rate limiting. It also implies that ACE activity is necessary for continual replenishment of rapidly inactivated ANG II. The possibility of rapid ANG II turnover is supported by the observation that ANG II is stoichometrically formed from plasma ANG I and will stimulate vasoconstriction during a single transit through the perfused systemic circulation of trout (Olson et al., '94). Thus both activation and inactivation

TABLE 4. Calculated half-time (in min) for clearance of radiolabelled catecholamines from the circulation

Species	Label	Fast $t_{½}$	Slow $t_{½}$	Reference
S. acanthias	^{3}H-Epi	5.8	234	Ungell and Nilsson ('83)
G. morhua	^{3}H-Epi	1.9	130	Ungell and Nilsson ('79)
O. mykiss	^{3}H-Epi	1.8	204	Nekvasil and Olson ('86)
O. mykiss	^{3}H-Nepi	1.7	230	Nekvasil and Olson ('86)
O. mykiss	^{14}C-Sucrose	1.8	240	Nekvasil and Olson ('86)
O. mykiss	^{3}H-Epi	2.9	433	Gamperl and Boutilier ('94)
O. mykiss	^{14}C-Sucrose	3.3	1364	Gamperl and Boutilier ('94)
P. marinus	Epi, Nepi[2]	4		Dashaw and Epple ('83)

[1]Calculated from figure.
[2]Unlabelled.

of ANG II, like that of catecholamines, appears to be very rapid.

Unlike catecholamine metabolism, however, the capacity for ANG II inactivation by trout may be saturated if ANG II levels are elevated for a prolonged period, or if ANG II vasoconstriction decreases blood flow to a tissue that normally is important in ANG II catabolism. The latter scenario has been proposed to occur in the gill where metabolism of ANG II is limited to the alamellar, filamental circulation (Olson et al., '86) and perhaps this occurs systemically as well. Nevertheless, it is evident that ANG II turnover is quite rapid and that the renin angiotensin system is a rapid on-off effector of arterial blood pressure in trout.

A single bolus of trout bradykinin (tBK) produces a complex, triphasic pressor, depressor, pressor response when injected in unanesthetized trout (Olson et al., '97). When the last two phases are blocked with indomethacin, tBK produces a single pressor phase whose recovery $t_{½}$ is consistent with convective dilution of the peptide and not an enzyme-limited process. Furthermore, the tBK recovery $t_{½}$ is not affected by ACE (kininase II) inhibition (Olson, unpublished observation), implying that peptidases other than ACE are sufficient for BK inactivation. Perhaps these same peptidases also are responsible for ANG II inactivation described above.

The rate of AVT inactivation, in comparison to other pressor hormones, is a slow process. It was not practical in these experiments to infuse AVT, however, with a recovery $t_{½}$ of 20–30 min (Table 2) it is clear that convection is not a factor in AVT inactivation. Furthermore, the recovery $t_{½}$ of isolated vessels in vitro was around five times faster than in vivo recovery (Tables 2, 3). This in effect rules out smooth muscle metabolic and mechanical processes as rate limiting factors and indicates that recovery time is dependent on removal of AVT or AVT-induced pressor substances from the circulation.

Le Mevel et al. ('93) noted that the effects of intra-arterial AVT injection in trout persisted longer than ANG II injection, which is consistent with our findings. It is possible that the dose injected into trout in the present study, and by Le Mevel et al. ('93), overloaded the metabolic capacity of the fish. Alternatively, it is possible that inactivation rate is low in trout to accommodate a low pituitary secretory rate or to insure that sufficient AVT reaches the effectors for tonic stimulation.

The relative contribution of individual tissues to inactivation of circulating biomolecules can be estimated form the tissue-specific rate constant (k_{Tiss}) according to the equation:

$$k_{Tiss} = -\ln[1 - (f_i \cdot f_p)] \cdot (CO/V_b) \qquad (5)$$

Where; f_i is the fractional inactivation of the biomolecule in a single transit through the tissue, i.e., the ratio of the molecule's concentration in venous to arterial blood; f_p is the fractional perfusion of the tissue, i.e., the ratio of tissue blood flow to cardiac output; CO is cardiac output; and V_b is effective blood volume (plasma volume if the hormone is not dissolved in red cell water). This relationship was previously described for the gill (Olson, '97) where f_p is 1.0 because the gill receives the entire cardiac output. It can also be assumed that the CO/V_b ratio is around 1.0 (Olson, '97). Figure 5 illustrates the relationships between $t_{½}$, f_i, and f_p (also see below).

Equation 5 and Figure 5 permit several predictions regarding hormone inactivation processes. First, the tissue with the largest rate constant (shortest $t_{½}$) will have the greatest impact on the rate of inactivation. Because no systemic tissue receives more than ~20% of the cardiac output (Olson, '92), the well perfused gill has a five-fold advantage over any other individual tissue based on flow alone. Thus a gill that only inactivates 20% of a hormone during a single transit ($f_i = 0.2$) will be as effective as any other tissue that inactivates all the hormone ($f_i = 1.0$) but only receives 20% of the cardiac output. However, if several tissues have similar f_i, then their f_p become additive and their collective contribution to inactivation is increased.

Second, the inactivation efficiency of a tissue, or group of tissues, can be estimated if the plasma half-time and tissue perfusion are known. If the half-time of a hormone in the circulation is between 2 and 3 min, a gill hormone inactivation fraction between 20 and 30% could account for all hormone inactivation. However, as shown in Figure 5B, a 2 min plasma $t_{½}$ can not be achieved by a tissue that receives ≤ 20% of the cardiac output and a 3 min $t_{½}$ will occur in a tissue receiving 20% of cardiac output only if inactivation is 100%. When a tissue receives 20% of the cardiac output, around 55% inactivation is required to produce a plasma half-time of 6 min (Fig. 5B). Unfortunately, there is little information on the $f_i \cdot f_p$ product for tissues other than gill (Olson, '97).

Third, if gill inactivation is greater than 20–30%, the relationship between cardiac output and plasma volume becomes the rate-limiting process

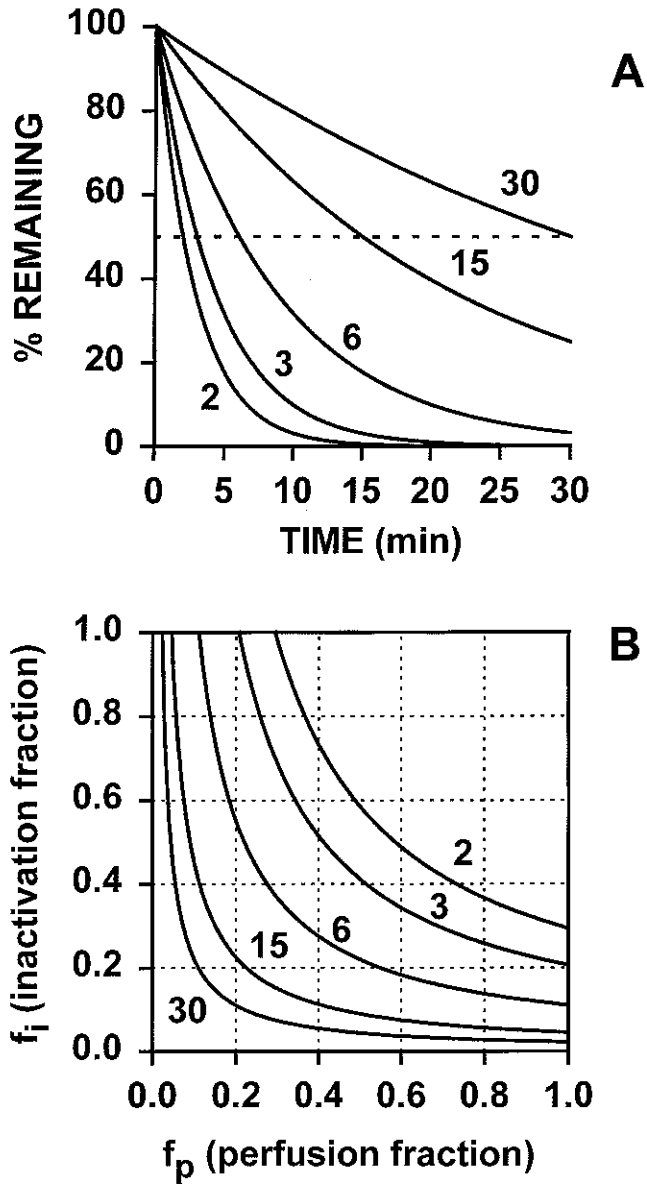

Fig. 5. Relationships between the plasma hormone clearance, fractional hormone extraction, and fractional perfusion. **A:** The predicted percent hormone remaining in the plasma as a function of time for plasma half-times $t_{½}$ of 2, 3, 6, 15, and 30 min (assuming no additional secretion). Curves with $t_{½}$ of 2 and 3 min are in the range of convective mixing. **B:** Relationship between fractional hormone inactivation by a tissue (f_i) and fractional perfusion (f_p) relative to cardiac output for $t_{½}$ of 2, 3, 6, 15, and 30 min. Example: if a hormone is inactivated only by gill (f_p = 1,0) then gill inactivation fraction will need to be 0.11 to produce a plasma $t_{½}$ of 6 min, whereas inactivation by a systemic tissue receiving 20% of the cardiac output (f_p = 0.2) will have to be 55% (f_i = 0.55) to achieve the same half-time.

for hormone inactivation. If the hormone rapidly distributes into the interstitial fluid, the total distribution volume will exceed the blood volume (V_b) and the rate constant calculated from plasma or volume will over-estimate the actual rate constant during periods of tonic hormone secretion or constant infusion. For example, if the total distribution volume is twice the plasma volume, then the rate constant will be half and the $t_{½}$ will be twice that predicted from Equation 5. In other words, if gill inactivation is 20% and the distribution volume is twice plasma volume the $t_{½}$ for the hormone will be slightly greater than 6 min. At this distribution volume (twice plasma volume), the $t_{½}$ will be restored to around 3 min if gill inactivation increases from 20 to 35%. If the molecule rapidly distributes throughout the entire extracellular fluid compartment (~200 ml/kg), gill inactivation would have to be 90% for a 3 min plasma $t_{½}$. However, dilution into the interstitial compartment will only have a significant effect on the rate constant and $t_{½}$ if it is nearly as fast as convective mixing of plasma and if the hormone can re-enter the circulation from the interstitial compartment as fast as it left. This information is not presently available.

Fourth, metabolic clearance of a hormone from plasma can be described by a single rate constant. It is evident from Equation 5 that either a single tissue with a large $f_i \cdot f_p$ product, or multiple tissues with similar $f_i \cdot f_p$ products, will produce a single rate constant that describes hormone inactivation kinetics. This also means that if multiple rate constants are present, factors other than tissue extraction/metabolism are operative. These factors can include; 1) reflux from an extravascular distribution volume or binding site, 2) production and subsequent clearance of radiolabelled metabolites of the injected hormone, or 3) uptake, storage and delayed secretion of unmetabolized hormone. Uptake, storage, and release of unmetabolized norepinephrine has been demonstrated to occur in the trout gill (Colletti and Olson, '88) and systemic circulation (Xu and Olson, '93) and may account for the second, long $t_{½}$ observed by many investigators after injection of labeled catecholamines in fish (Table 4).

It is evident from these studies that physiological recovery from circulating vasoactive catecholamines, and the peptides, ANG II and bradykinin is on the same timescale as is physiological activation following hormone secretion. Because both activation and inactivation are convection-limited processes, it can be concluded that these hormones

are rapid on/off effectors of cardiovascular function in trout. This is probably the case in other teleosts as well.

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