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RAPID COMMUNICATION

Divergence in Vascular Actions of Prostacyclin During Vertebrate Evolution

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ABSTRACT   Prostacyclin (PGI$_2$) generation by teleost and elasmobranch fish vascular wall was detected, as 6-keto-PGF$_{1\alpha}$-like immunoreactivity, in the incubation medium of ventral aorta rings from *Anguilla anguilla, Conger conger* and *Scyliorhinus stellaris*. The average yields after 15 min incubation were the following (ng of 6-keto-PGF$_{1\alpha}$-like/g of wet tissue): *A. anguilla* = 116.8$\pm$30; *C. conger* = 7.50; *S. stellaris* = 58.0$\pm$10.

Accordingly, the vascular responses to synthetic PGI$_2$ were examined on the isolated and saline-perfused head of four teleost (*A. anguilla, C. conger, Scorpaena porcus* and *Solea solea*) and two elasmobranch (*S. stellaris* and *Torpedo marmorata*) species. In teleosts, PGI$_2$ gave a dose-related increase in overall gill vascular resistance, unaffected by indomethacin, phenoxybenzamine and phentolamine pretreatment or by previous decerebration of the animal. Conversely, in elasmobranchs PGI$_2$ elicited a diphasic response, characterized by a transient, not dose-related constriction, followed by a longer lasting and dose-dependent dilation.

The effects of PGI$_2$ on isolated branchial arch (in *A. anguilla, C. conger* and *S. stellaris*) and ventral aorta strips (in *C. conger* and *S. stellaris*) were also examined. Both preparations shared the same responsivity of the head *in toto*, confirming the divergence observed in this respect between teleosts and elasmobranchs. The reactivity of teleost branchial vessels to this autacoid appears to be unique among the other vertebrates examined thus far.

In mammals, PGI$_2$, the major metabolite of arachidonate (AA) in large vessel wall, is a potent vasodilator and the strongest endogenous inhibitor of platelet aggregation known. A physiological antagonism to thromboxane A$_2$ (another component of the prostanoid system, mainly generated by platelets) with respect to effects on vascular tone and platelet aggregability has been proposed (Moncada and Vane, '79). Similar vasoactive properties have been described in the amphibian *Rana catesbeiana* (Leffler et al. '80; Herman et al. '82). The aim of our experiments was to investigate both the occurrence of PGI$_2$ biosynthesis from endogenous AA stores and the vascular actions of this autacoid on different segments of teleost and elasmobranch fish branchial circulation.

MATERIALS AND METHODS
Fish.

S. *stellaris* (1,000–3,000 g), *T. marmorata* (200–400 g) and *C. conger* (500–1,500 g) were obtained during winter and spring from the Aquarium of the Zoological Station of Naples where they were kept in holding tanks with circulating seawater for at least ten days at 16–18°. *A. anguilla* (90–180 g), *S. porcus* (400–500 g) and *S. solea* (200–250 g) were obtained from local dealers. The fish were kept at 16–18° either in circulating tap water or seawater until used.

Solutions and substances

Salines were prepared according to Young ('33). Seawater teleost Ringer: NaCl 13.5 g/

KCl 0.6 g/l; $CaCl_2$ 0.25 g/1; $MgCl_2$ 0.25 g/l; $MgCl_2$ 0.35 g/l. Freshwater teleost Ringer: NaCl 5.5 g/l; KCl 0.14 g/l; $CaCl_2$ 0.12 g/l. Elasmobranch Ringer: NaCl 22 g/l; KCl 0.52 g/l; $Cacl_2$ 0.44 g/l;$MgCl_2$ 0.47 g/l;urea 29 g/l. PGI_2 (Wellcome Research Laboratories, United Kingdom) was prepared in saline just before injection from stock solutions in tris buffer (pH8.4) stored at -20°. Indomethacin, Phentolamine, Phenoxybenzamine, Atropine and Mepiramine were purchased from the usual commercial sources. 6-keto-$PGF_{1\alpha}$ (Wellcome) and ^{3}H-6-keto-$PGF_{1\alpha}$ (Radiochemical Center, Amersham, England) were also used.

Incubation and prostanoid extraction

The animals were sacrificed by decapitation and the ventral aorta was excised. The artery was carefully freed from surrounding tissue and cut into rings. The tissues were incubated for 15 min in a metabolic shaker at 18°, 0.5 ml of saline were added to ventral aorta rings. After incubation, the medium was acidified to pH 3.0 with 0.5 M citric acid and 0.25 ml of saturated NaCl solution was added, twice extracted with an equal volume of ethylacetate and evaporated. The residue was dissolved in 1 ml of 50 mM buffer (pH 7.4) and used for radioimmunoassay determination of 6-keto-$PGF_{1\alpha}$.

Radioimmunoassay

RIA of 6-keto-$PGF_{1\alpha}$ was performed according to Salmon and Flower ('79).

Isolated and perfused head

Perfused head was prepared according to Payan and Matty ('75) with minor modifications. The heart was exposed, heparin (500 I.U./100 g) injected into the ventricle and allowed to flow through the gills. After having tied the sinus venosus, the fish was decapitated just behind the pectoral fins and a polyethylene catheter inserted into the ventral aorta. The head was then put in a plastic box filled with circulating water at constant temperature. During preparation and experiment gills were ventilated either with filtered tap water or seawater (T: 18°; flow: 600 ml/min) continously bubbled with a stream of air. Perfusion was carried out by means of a peristaltic pump with a steady flow of 2.5 ml/100 g (Satchell '71). After this procedure, 10–15 min were allowed to reach a steady pressure before beginning the experiment. Cannulation of dorsal aorta was

also performed in some cases, but, apart from the expected rise in inflow pressure, no variation in the response was observed.

Isolated branchial arch

Branchial arch was prepared as follows (Bergman et al. '74). The gills were cleared of the blood by passing heparinized saline through the isolated head for 15 min; the gill arch was dissected out of the branchial basket and a cannula inserted into the afferent branchial vessel. Position of ligature was such that approximately 80% of the filaments were perfused. Each gill arch was suspended in a bath with conditions similar to those described for the isolated head. The pump output to each arch was defined assuming an equally divided flow to all gill arches and considering that only 80% of the arch was perfused.

Pressure measurements and calculations

The inflow pressure both in isolated head and branchial arch was measured with a Statham P23 pressure transducer connected to a Beckmann polygraph. The overall gill vascular resistance (Nilsson '84) was calculated (Burton '72) from $Rg = \delta P/Q$. Where Rg = gill vascular resistance; δP = drop in pressure through the gills; Q = perfusion flow (ml/min/100 g).

Ventral aorta strip

The ventral aorta was dissected out and freed of surrounding tissue. The artery was then cut spirally and suspended in an organ bath containing an air-bubbled saline at room temperature (18°). All preparations were left suspended in saline for at least 1 hr before experiments were begun. Preparations were exposed to substances for three min. Responses were measured by means of an isotonic transducer connected to a recorder (Basile).

RESULTS

Ventral aorta rings of freshwater adapted *A.anguilla* and seawater *C.conger* and *S.stellaris*, when incubated in an appropriate saline at room temperature (18–20°) for 15 min, produced detectable amounts of 6-keto-$PGF_{1\alpha}$-like immunoreactivity in the absence of any stimulation. The average yields obtained in the three species were the following (ng/g of wet weight): *A.anguilla*=116.8±30 (n=4); *C.conger*=7.50 (n=2); *S.stellaris* =58.0±10 (n=4). In interpreting these

results, a 30% crossreactivity of 6-keto-PGF$_{1\alpha}$ with 6-keto-PGF$_{2\alpha}$ should be taken into account (L. Parente, personal communication).

PGI$_2$ administered in bolus injections ranging from 10 ng to 10^4 ng/100 g produced a dose-related vasoconstriction in the isolated and perfused head of all the teleost species examined (Fig. 1). This effect was not inhibited by indomethacin ($1.0* \ 10^{-5}$M), phenoxybenzamine ($1.0*10^{-6}$M) or phentolamine ($1.0*10^{-5}$M) pretreatment or by previous me-

chanical decerebration of the animal (data not shown). Vehicle alone (phosphate buffer, pH 8.4) did not affect inflow pressure. Conversely, in the elasmobranch, S.stellaris and T.marmorata, a dilation preceded by a transient, not dose-related constriction was observed (Fig. 1). As shown in Figure 2, the action of PGI$_2$ on gill arch preparation of A.anguilla and C.conger was consistent with the one observed in the head in toto. A dose-dependent increase in vascular resistance followed PGI$_2$ administration at doses as low
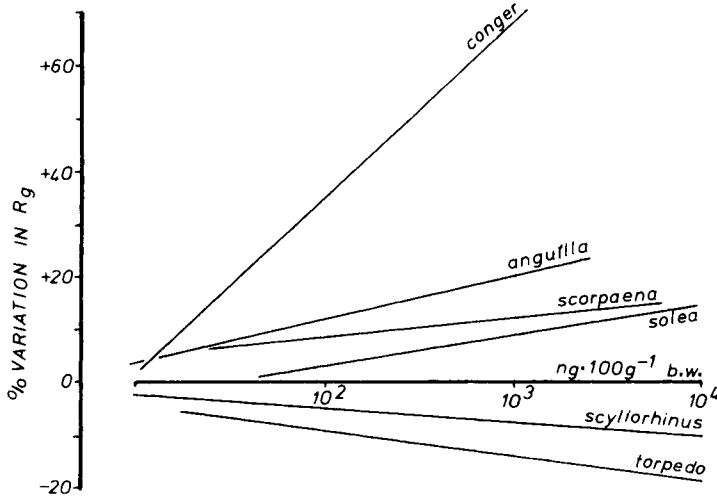

Fig. 1. Isolated and saline-perfused head: bolus injections of PGI$_2$ (ranging from 10 to 10^4 ng/100 g) caused a dose-dependent vasoconstriction in teleosts. In elasmobranchs, a dilation was evoked: the preceding constrictory phase, transient and not dose-related, has been omitted in figure. Results are expressed as percent variation in gill vascular resistance (Rg) vs. ng of PGI$_2$/100 g. Control Rg was 22.7 ± 2 (n=5). Experimental number: 3–8 animals.
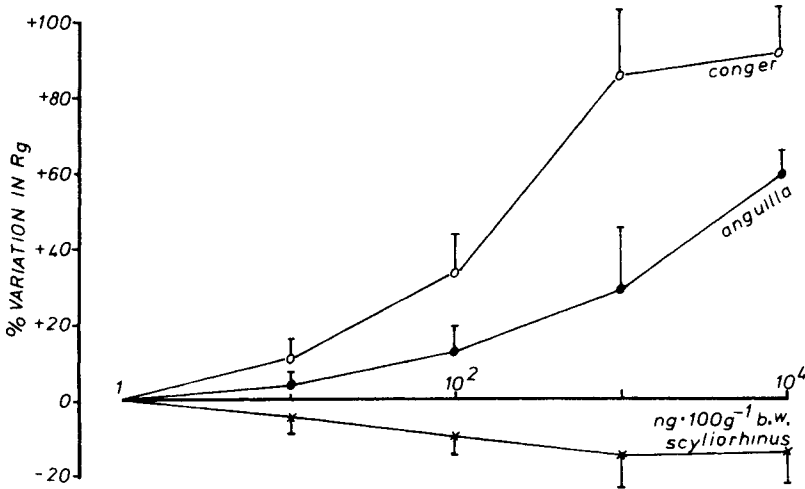

Fig. 2. Isolated-perfused branchial arch: the vascular responses to PGI$_2$ were compared in two teleosts and one elasmobranch. Results are expressed as in Figure 1. Vertical bars: S.E.M. Experimental numbers: C. conger=8; A.anguilla and S.stellaris=4.

as 10 ng/100 g. Similarly, in isolated arches from S. stellaris the typical pattern constriction-long-lasting dilation was reproduced (Fig. 2).

Changes in tension of spiral ventral aorta strips from C. conger and S. stellaris induced by PGI_2 were also examined. The effects of PGI_2 on arterial smooth muscle preparations of each species paralleled those observed in branchial vasculature (Fig. 3). A concentration-related contraction was elicited in C. conger (threshold = 100 ng/ml of bath), whereas a relaxation (preceded by a brief contraction) occurred in S. stellaris (threshold = 10 ng/ml of bath). Furthermore, neither effect was inhibited by a mixture of antagonists (Gilmore et al '68) of the following composition: methysergide maleate $(2.7*10^{-7}$ g/ml), propopranolol HCl $(2.6*10^{-6}$ g/ml), atropine sulfate $(10^{-7}$ g/ml) and indomethacin $(2*10^{-6}$ g/ml).

DISCUSSION

From the findings here reported it appears that teleost and elasmobranch vessel walls are both able of producing PGI_2 using endogenous AA as a substrate and of responding
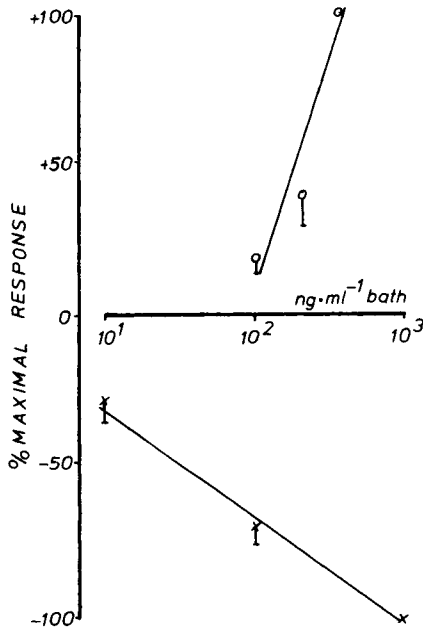

Fig. 3. Isolated ventral aorta strip: in C. conger (upper panel) PGI_2 contracted the arterial preparation (threshold: 100 ng/ml of bath), whereas in S. stellaris (lower panel) after a brief, not dose-dependent contraction, it produced a longer lasting relaxation (threshold: 10 ng/ml of bath). Results expressed as mean percent of the maximal response. Vertical bars: S.E.M. Experimental number = 4.

in a dose-related fashion to the administration of this autacoid. In our laboratory, generation and vascular activities of other prostanoids and prostanoid precursors in fish circulation are in study (Piomelli et al., in preparation). We were able to show that teleost and elasmobranch branchial vasculature can convert exogenous AA to vasoactive products of cyclo-oxygenation, and that synthetic prostaglandins ($PGF_{2\alpha}$ and PGE_2) can elicit an array of responses in the perfused head, branchial arch and spiral aortic strip of several fish species. Furthermore, we observed that the vasopressor response that follows the administration of angiotensin II to the isolated head of the freshwater adapted eel, A. anguilla, can be blocked by the cyclo-oxygenase inhibitor indomethacin (Piomelli and Pinto, submitted). These results, taken together, may lend support to the hypothesis of a role of eicosanoids in teleost and elasmobranch gill circulation.

In this frame, the observation of a divergence in the vascular activity of PGI_2 between teleost fish, on one hand, and elasmobranchs, amphibians and mammals, on the other, may be of interest. We have attempted to rule out reflex responses and mediation via some other transmitters by the use of decerebrated animals, isolated tissues (as opposed to in toto perfused head) and various pharmacological antagonists.

Even though these experiments do not clarify the mechanism of PGI_2-induced vasoconstriction in teleost fish, they clearly suggest a local site of action, which is consistent with the finding of a local production of this humor by the vessel. Two characteristics of teleost responsivity to PGI_2, as they appear from our results, are worthy of being mentioned. First, its apparent group-specificity: within teleosts, species phylogenetically distant showed a qualitatively similar response pattern. Second, the lack of a direct relationship with any particular environmental conditions. In fact, in spite of the diversity of habitat and behaviour, the four teleost species studied here shared a common responsivity to PGI_2. Consistently, teleost and elasmobranch species dwelling in the same areas and showing related behavioural patterns (such as T. marmorata and S. porcus) were different in this respect. It seems reasonable therefore to suggest that the divergence observed here is not immediately explainable in terms of a specific environmental need of modern teleosts. More subtle changes in the homeostatic control of the milieu interieur

might have occurred in this subclass to give the basis of such a divergence.

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