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Pharmacological Properties of the Adrenergic Receptors Regulating Systemic Vascular Resistance in the Rainbow Trout

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Summary. 1. The adrenergic receptors in the systemic vasculature of the rainbow trout have been pharmacologically characterized using an isolated trunk preparation perfused at constant flow.

2. The dominant α-constrictory receptors in the trunk are similar to those of mammals in their adrenaline/noradrenaline potency ratio (3.2/1.0), and in the natures of their blockade by phenoxybenzamine and yohimbine. However they are more selective than mammalian α-receptors, responding directly to only adrenaline and noradrenaline, and not to phenylephrine, methoxamine, dopamine, or isoprenaline.

3. Tyramine and dopamine cause weak α-adrenergic constriction, apparently indirectly through the release of catecholamine stores.

4. The α-adrenergic response is susceptible to inhibition by competitive β-blocking agents, but this effect is due to non-competitive antagonism with a point of action beyond the adrenergic receptor.

5. β-dilatory receptors of the β_2-variety, as in the homologous systemic vasculature of mammals, also apparently occur, but their dilatory actions cn only be demonstrated against a background of α-adrenergic vasoconstriction.

6. The racemate d,l-isoprenaline is a more potent vasodilator than the pure isomer l-isoprenaline during α-adrenergic tone because of the competitive α-blocking activity of the d-isomer.

Introduction

Adrenergic mechanisms play an important role in the regulation of vasomotor tone, and therefore vascular resistance, in the systemic circulation of higher

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Abbreviations: R_s = systemic vascular resistance, AD = adrenaline, NAD = noradrenaline, ISO = isoprenaline, PHE = phenylephrine

vertebrates. The net level of adrenergic tone results from the summated excitatory (vasoconstrictory) effects of α-receptor stimulation and inhibitory (vasodilatory) effects of β-receptor stimulation. In teleost fish, the presence of β-adrenoreceptors in the branchial vasculature and of α-adrenoreceptors in the systemic vasculature has now been documented (Reite, 1969; Rankin and Maetz, 1971; Stevens et al., 1972; Helgason and Nilsson, 1973; Wood, 1974a, b; Wood and Shelton, 1975). Branchial α-receptors have also been found, at least in some species (Belaud et al., 1971; Bergman et al., 1974; Wood, 1975). It is not yet clear whether β-adrenoreceptors occur in the systemic vessels of teleosts, for there exists evidence both for (Chan, 1967; Helgason and Nilsson, 1973) and against (Randall and Stevens, 1967; Nilsson, 1972) their presence, while still other data is equivocal (Stray-Pedersen, 1970; Holmgren and Nilsson, 1974).

Pharmacologically, the β-receptors of the gills seem similar to the β_1-adrenoreceptors which occur in the homologous coronary vasculature of mammals (Wood, 1975). If a similar homology were to apply to the peripheral circulation, systemic β-receptors of the β_2-variety would be expected (Arnold, 1972), but nothing is known on this point. Except for the recent work of Holmgren and Nilsson (1974) on isolated coeliac artery strips, there similarly exists little information on the pharmacology of systemic α-receptors in fish.

The aim of the present study was therefore a thorough examination of the adrenergic receptors in the systemic circulation of a teleost fish. Particular attention has been paid to detecting the possible presence of β-receptors and to characterizing the systemic adrenoreceptors with basic pharmacological techniques. The pump perfused trunk of the rainbow trout, *Salmo gairdneri*, proved very suitable for the investigation because of its ease of preparation, durability, and the demonstrated advantages of constant flow perfusion in vasomotor tone comparisons (Wood and Shelton, 1975).

Materials and Methods

Rainbow trout (80–400 g) were purchased from a commercial supplier in Holt, Norfolk, and maintained on a pellet diet in an outdoor tank under seasonal conditions. In an attempt to standardize the recent thermal history of the animals, all fish were held in the laboratory at 14.5 ± 1.5 °C in dechlorinated tapwater for 1–2 weeks prior to experimentation. During this period, the trout were not fed. Experiments were performed during all months of the year. Ninety-eight animals were used exclusively in the investigations of systemic adrenergic pharmacology reported here. Some additional data, chiefly on the seasonal variation in the response to adrenaline and the effects of adrenergic blocking agents on systemic vascular resistance, have been taken from a further 75 identical preparations used in other studies (Wood and Shelton, 1975; Wood, unpublished results). All experiments were conducted in a constant temperature room at 5 ± 1 °C.

I. The Preparation

A trout was anaesthetized in 650 mg/L MS-222 (Sandoz) on an operating table at the acclimation temperature and injected intraventricularly with 500 i.u. of sodium heparin (Sigma)/100 g. Five minutes later, the animal was transected at the level of the anterior end of the heart, the atrium and ventricle removed, and the dorsal aorta cannulated with PP 190 or 200 tubing (Portex). The trunk was flushed free of blood by perfusing through the dorsal aortic catheter at a head of 30 cm H_2O. The perfusion medium consisted of Cortland salmonid saline (Wolf, 1963) containing

10 i.u./ml of heparin (Evans Medical) and 4% polyvinylpyrrolidone (average molecular weight = 40,000; Sigma) at a pH of 7.4–7.5 (Wood, 1974a). The preparation was then immersed in a constant-level bath of Cortland saline at 5 ± 1 °C, and pump perfusion immediately commenced.

II. The Perfusion System

The perfusion equipment has been described in detail elsewhere (Wood and Shelton, 1975). In brief, a pressure independent peristaltic pump (Quickfit) impelled perfusate at constant flow (0.45–0.55 ml/100 g total body weight · min) through a flask containing a small air bubble (2–3 ml) for depulsation and then into the trunk via the dorsal aortic catheter. The reservoir of perfusion medium rested on a top-loading balance for flow measurement and was bubbled with oxygen throughout an experiment. Perfusion pressure was measured relative to the bath surface as zero with a Sanborn 267 BC pressure transducer connected via a T-joint between the depulsator and the trunk. A side-arm distal to the T-joint and immediately proximal to the point of cannulation was used for the injection of drugs into the perfusion line. Changes in the vascular resistance of the trunk (R_s; i.e. the systemic vascular resistance) were monitored as changes in the perfusion pressure at constant flow and calculated as described by Wood and Shelton (1975).

III. Drugs

(i) Agonists: l-adrenaline bitartrate (AD), l-noradrenaline bitartrate (NAD), l-isoprenaline bitartrate (l-ISO), d,l-isoprenaline hydrochloride (d,l-ISO), l-phenylephrine hydrochloride (PHE), tyramine hydrochloride, dopamine hydrochloride (all Sigma), d,l-nylidrin hydrochloride (Smith and Nephew), and d,l-methoxamine hydrochloride (Burroughs Wellcome). In a few experiments, various equivalent weights of potassium chloride were substituted for sodium chloride in the Cortland saline to produce high potassium perfusion media with unaltered total ionic concentration.

(ii) Antagonists: yohimbine hydrochloride, propranolol hydrochloride, dichloroisoproterenol hydrochloride (all Sigma), phenoxybenzamine hydrochloride (Smith, Kline, and French), and guanethidine sulphate (Ciba).

IV. Methodology

During the first 5–80 min of perfusion, the vascular resistance of the trunk spontaneously declined, eventually reaching a value (baseline R_s) which, with very minor variations, was stable for the remainder of the experiment (up to 24 h). Wood and Shelton (1975) have shown that this phenomenon reflects a loss of intrinsic α-adrenergic tone. The preparation was allowed to stabilize at baseline R_s before any experiments were begun.

Agonists were usually administered by injection as discrete doses (volume = 0.1 ml) through the side-arm into the perfusion line. A momentary spike on the pressure record marked the point of injection. Dose/response studies were carried out with a sequentially and logarithmically increasing dose order; each response was allowed to completely subside before the next injection was given. Antagonists, and in a few cases agonists, were added directly to the perfusion reservoir and were therefore administered as constant concentrations in the perfusate. Antagonists were allowed to act for at least one hour at the concentration stated (i.e. after passing through the dead space of the system) before agonists were retested in the continued presence of the antagonist. Because of the known lability of many of the drugs used, all solutions were stored in the dark at 1 °C during use and renewed approximately every 3 h.

V. Treatment of Data

Because maximal constrictory responses took many hours to subside and often caused decreased responsiveness to subsequent doses, it was not feasible to routinely determine complete dose/response

curves (from threshold to maximum). Consequently the work reported here deals with approximately the lower 50% of the complete dose/response relationship for adrenergic constriction. As the size of a response was positively, though loosely, correlated with the level of baseline R_s, responses have been computed as percentage changes of baseline R_s. Each preparation was used as its own control, dose/response curves being recorded both before and after an experimental treatment. Results have been expressed as means ± 1 S.E., and the significance of experimental treatments assessed by application of the paired Student's t-test (two-tailed) to responses at the same dose level. Because of this paired experimental design, some of the differences reported here are highly significant despite the proximity of the standard errors. Unless otherwise stated, where only the results of individual experiments are given, at least 3 trunks showed qualitatively identical results and no contradictory data were obtained. Where original perfusion pressure records are shown in Figures, 2–5 cm H_2O of the pressure was due to the resistance of the perfusion cannula in all cases.

Results

I. α-adrenergic Studies

The naturally occurring catecholamines AD and NAD act on both α- and β-adrenoreceptors in higher vertebrates. When injected into the perfusion line in the present preparation, both amines caused dose dependent vasoconstriction (Fig. 1). AD was slightly more potent than NAD (see p. 224). The doses of both agonists for threshold and maximum responses were approximately 320 pmoles–1 nmole and 320 nmoles respectively. Injections of the relatively specific β-adrenergic stimulant 1-ISO (up to 100 μmoles) and the relatively speci-

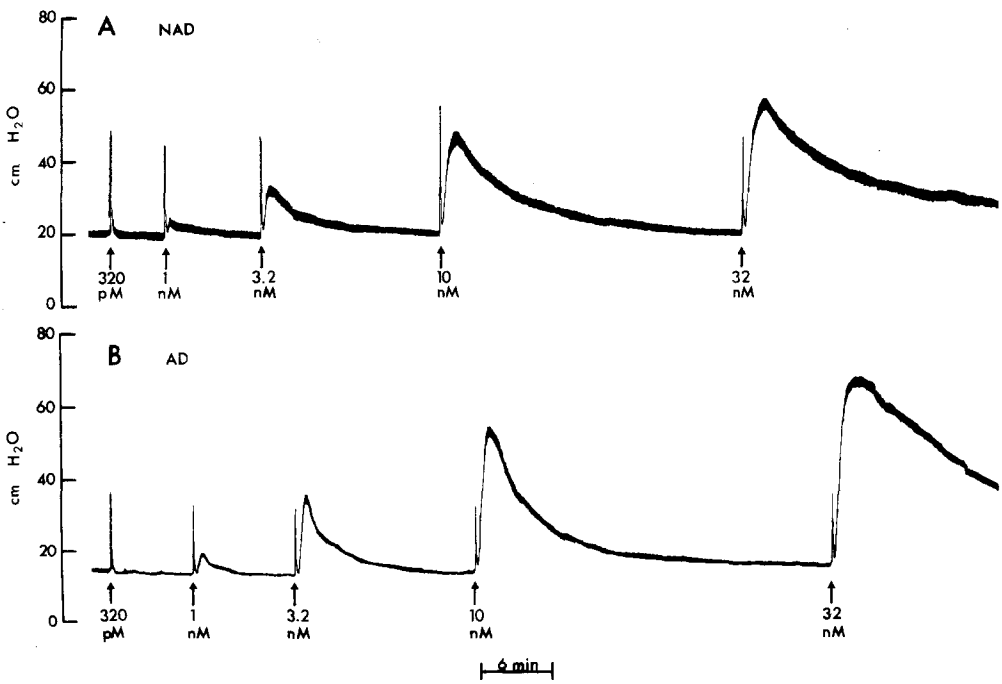

Fig. 1. Typical perfusion pressure records of constrictory dose/response curves to (*A*) NAD and (*B*) AD in two different trunk preparations

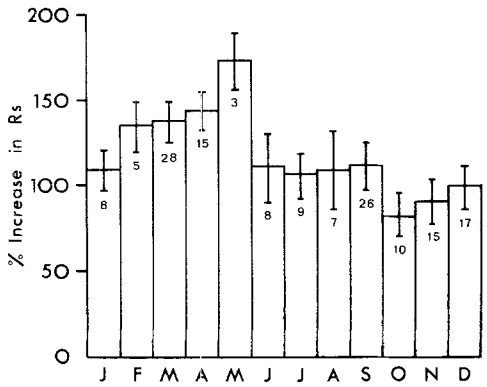

Fig. 2. Seasonal variation in the response to 10 nmoles AD in the trunk preparation. Means ±1 S.E. (*N*). A single line underscores monthly means between which it has not been possible to demonstrate significant differences at the 5% protection level by Kramer's (1956) modification of Duncan's Multiple Range Test (Duncan, 1955)

$F=1.99$ $p<0.05$

Oct. Nov. Dec. July Jan. Aug. June Sept. Feb. Mar. Apr. May

fic α-adrenergic stimulant PHE (up to 100 μmoles) were without definite effect, even when the highest dose was administered first to negate any progressive desensitization. However, it should be noted that in 2 of 5 experiments, a slight, very slow rise in R_s occurred after 100 μmoles PHE. This perhaps represented the non-specific response previously noted at such very high dose levels of PHE (Wood and Shelton, 1975).

The actual size of the response to a given dose of AD or NAD was quite stable during an experiment in an individual trunk but varied widely between preparations at different times of the year. A definite seasonal pattern emerged (Fig. 2). The average response to 10 nmoles AD was smallest in October, steadily increased over the winter to a value approximately twice as large in May, and then dropped to a lower level over the summer months. This variation was statistically substantiated, the mean responses in March, April and May being significantly greater ($p<0.05$) than those in October and November. A similar analysis revealed no change in baseline R_s over the year, thereby eliminating this factor as a cause of the phenomenon. As the fish had been isolated from the normal external environment for 1–2 weeks, at least the short term effects of temperature, photoperiod, and feeding history would seem to be unimportant in its genesis. A possible correlation occurred with the reproductive cycle. Trout first showed pronounced gonadal development in October and November when the response was smallest, and breeding terminated in April and May when the response was greatest.

Phenoxybenzamine (10^{-5} M in the perfusate), a highly potent non-equilibrium antagonist of α-adrenoreceptors (Nickerson, 1970), completely abolished the response to AD (Fig. 3A), even at injection doses of AD up to 100 μmoles (2 experiments). Yohimbine (10^{-5} M), a much less potent but highly selective

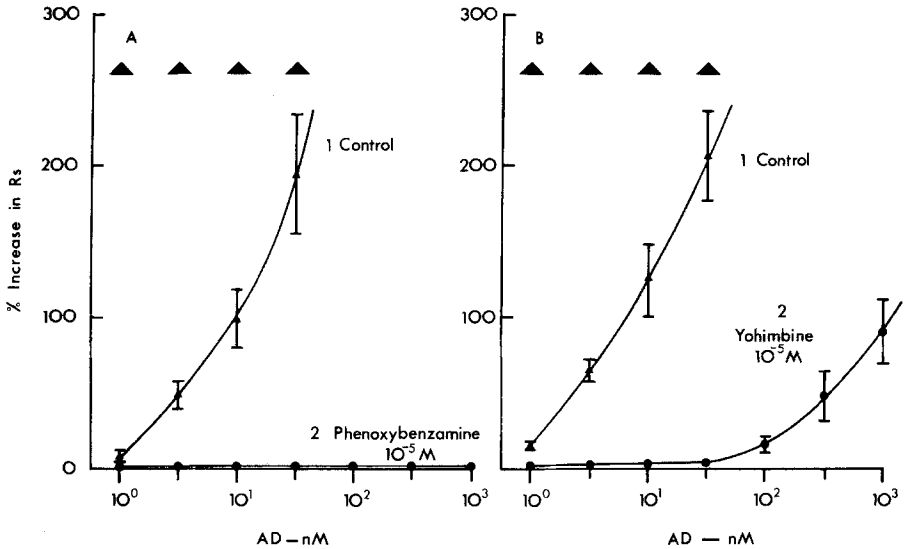

Fig. 3. The effects of the α-adrenergic antagonists (*A*) 10^{-5} M phenoxybenzamine ($N=6$) and (*B*) 10^{-5} M yohimbine ($N=6$) on the constrictory dose/response curve to AD. Means ±1 S.E. Large triangle = $p<0.05$. 1, ▲ control; 2, ● after α-blocker

α-adrenergic blocker of the competitive type (Boyd et al., 1962; Sheys and Green, 1972) produced a surmountable blockade of the AD response with an approximately parallel shift of the injection dose/response curve (Fig. 3A). AD-mediated dilation after α-adrenergic blockade was never observed. Two competitive β-adrenergic blocking agents of relatively good selectivity, propranolol and dichloroisoproterenol (Moran, 1967; Nickerson, 1970), were without significant effect on the injection dose/response curves to AD at the same concentration as the α-blockers (10^{-5} M) (Fig. 4A, B). However it should be noted here that in other experiments, the β-antagonists, especially at higher concentrations, did interfere significantly with responses to AD, a point which will be dealt with in Section II of Results. The four agents at 10^{-5} M influenced injection dose/response relationships to NAD in the same fashion as those to AD in Figures 3 and 4. Thus, at least in the present experiments, their actions on the systemic α-receptors of trout were similar to those on mammalian vascular α-receptors.

In light of the inactivity of the selective α-adrenergic agonist PHE (up to 100 μmoles) in the trout trunk, another compound of similar properties was tested. d,l-Methoxamine is an even more specific α-adrenergic stimulant than PHE in mammals (Innes and Nickerson, 1970). It was completely inactive on the trout preparation in injection doses up to 10 μmoles and as a constant concentration in the perfusion medium up to 10^{-2} M, the highest levels tested.

1-ISO is a powerful β-stimulant with slight but significant α-activity on mammalian α-receptors (Jenkinson, 1973). To test whether its failure to produce α-constriction in the systemic vasculature of the trout was due to over-riding

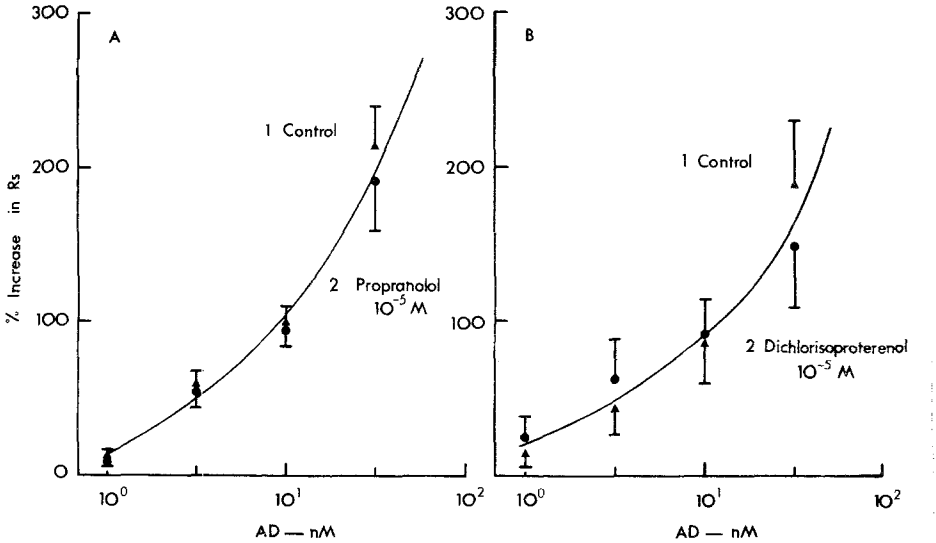

Fig. 4. The effects of the β-adrenergic antagonists (*A*) 10^{-5} M propranolol ($N=5$) and (*B*) 10^{-5} M dichloroisoproterenol ($N=6$) on the constrictory dose/response curve to AD. Means ±1 S.E. No significant differences. 1, ▲ control; 2, ● after β-blocker

β-dilation, 1-ISO (up to 100 μmoles) was injected after pretreatment with dichlorisoproterenol or propranolol (10^{-5} M). Its inactivity persisted.

Tyramine is a sympathomimetic amine of totally indirect action in mammals, producing adrenergic responses only by displacing and releasing tissue catecholamine stores (Trendelenburg, 1963). When injected into the perfused trunk, this drug produced a small vasoconstriction (5–30% increase in R_s) which developed slowly over several minutes (cf. Fig. 5). Successive doses of tyramine caused a marked tachyphylaxis to itself but not to AD (cf. Fig. 5), a typical action of an indirectly acting agent. Because of this phenomenon, it was not possible to construct dose/response curves or accurately determine sensitivities, but the threshold dose was usually in the range 1–10 nmoles. Tachyphylaxis similarly complicated blocking studies, but it was evident that the response persisted after the β-antagonists propranolol or dichloroisoproterenol (10^{-5} M), but not after the α-antagonists yohimbine or phenoxybenzamine (10^{-5} M).

Dopamine is the biosynthetic precursor of NAD, exerting α-activity, β-activity, indirect activity, and some specific dopaminergic activity in mammals (Goldberg, 1972). When injected into the trunk, the amine produced a slow constrictory effect similar to that of tyramine with approximately the same threshold (1–10 nmoles) (Fig. 5). Tachyphylaxis was again prominent (Fig. 5), and as with tyramine the response was blocked by the two α-blocking agents but not by the two β-blocking agents at 10^{-5} M. Rapid constriction, such as that caused by AD and NAD, or dilation of any sort was never observed. In both of two experiments in which this was tested, dopamine and tyramine produced crossed-tachyphylaxis to each other (Fig. 5).

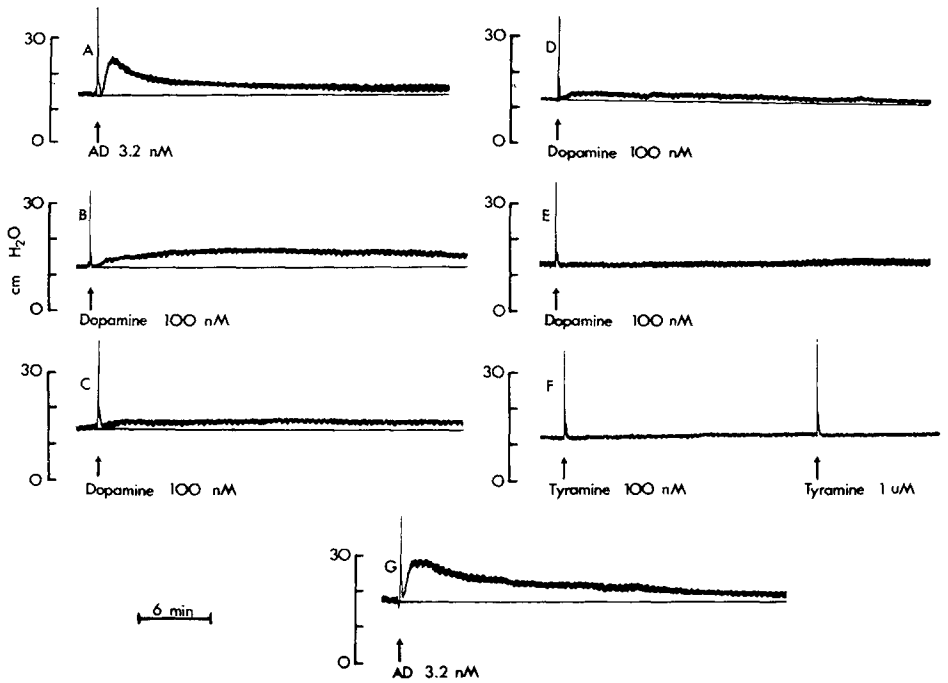

Fig. 5A–G. Typical responses to dopamine injections in a single trunk preparation showing progressive tachyphylaxis, and crossed-tachyphylaxis with tyramine but not with AD. (A) The initial response to 3.2 nmoles AD; (B), (C), (D), and (E) the declining response to successive injections of 100 nmoles dopamine [two further injections of 100 nmoles dopamine intervened between (D) and (E)]; (F) the lack of effect of 100 nmoles and 1 μmole tyramine after (B)–(E); (G) the persistence of the responses to 3.2 nmoles AD after (B)–(F). Baselines representing the pre-injection perfusion pressures have been inserted

II. β-adrenergic Studies

The failure of 1-ISO to produce dilation in the preceding experiments could be due to an already maximal relaxation of the resistance vessels at baseline R_s rather than to a lack of β-adrenoreceptors in the systemic vasculature. If this were the case, β-dilation by 1-ISO should be clearly demonstrable after raising R_s through a different receptor mechanism. Unfortunately, stimulation of cholinergic constrictory receptors proved unsuitable for this purpose because the level of vasoconstriction quickly decayed (Wood, unpublished results). The inactivity of the selective α-agonists PHE and d,l-methoxamine similarly eliminated pure α-receptor stimulation. Instead it was necessary to create tone by the use of AD or NAD, both of which have significant β-stimulating potential. Therefore if β-dilatory receptors are present in the systemic vasculature of the trout, the level of vasomotor tone produced by AD or NAD will already be the net result of summated α-(contracting) and β-(relaxing) effects. Nevertheless, early studies of mammalian aortic smooth muscle were able to reveal β-dilation by isoprenaline in preparations contracted by AD or NAD (e.g. Furchgott and Bhadrakom, 1953).

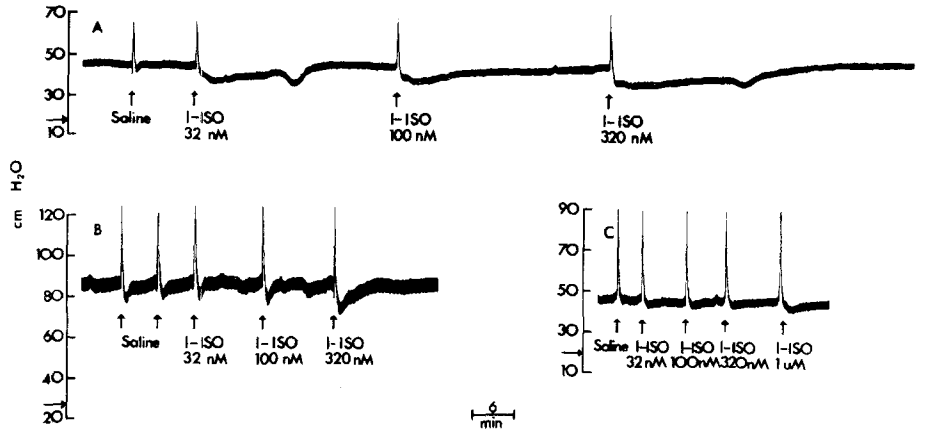

Fig. 6A–C. Responses to injections of l-ISO in three trunk preparations previously constricted by 5.5×10^{-6} M AD. Horizontal arrows indicate perfusion pressure at baseline R_s before constriction by 5.5×10^{-6} M AD. Examples of: (A) well defined dilatory responses to l-ISO (note poor dose dependency); (B) poorly defined responses to l-ISO (note similar dilations caused by saline injections); (C) virtual lack of effect of l-ISO

The following results were essentially the same whether AD or NAD was employed as the α-constrictor; the former was used in most trials. At 5.5×10^{-6} M in the perfusate, a level which lies approximately in the middle of the systemic concentration/response relationships (Wood and Shelton, 1975), AD produced a relatively stable increase in R_s ($344.9 \pm 16.2\%$ ($n = 31$) of baseline R_s) (e.g. Fig. 7A, C) against which to observe possible β-effects. In about half of the preparations, injections of l-ISO of 10 nmoles or greater caused a definite vasodilation from this level, though there seemed to be poor dose dependency; control injections of saline vehicle had almost no effect (Fig. 6A). However in other trunks, saline injections produced dilations similar to those caused by l-ISO (Fig. 6B; presumably a "viscous" response of the vascular muscle to the "stretch" of the injection pressure surge). In still others, the β-agonist was virtually without effect (Fig. 6c). In those preparations showing a clear response to l-ISO, the β-antagonist propranolol (10^{-5} M in the perfusate) diminished or abolished the dilation; however this result was inconclusive because propranolol (10^{-5} M) simultaneously reduced the level of tone by 5–60% (e.g. Fig. 7d), despite its lack of significant inhibitory effect at this concentration on the injection dose/response curve to AD (Fig. 4A).

In order to overcome the problem of injection artifact, isoprenaline was added directly to the perfusion medium in various concentrations during tone created by 5.5×10^{-6} M AD or NAD. In the interest of economy, d,l-ISO was initially used in these experiments in place of the more expensive pure isomer l-ISO; however important differences in their modes of action later became apparent, so the two compounds were studied separately. Both d,l-ISO and l-ISO (thresholds: $10^{-6} - 10^{-5}$ M) produced a stable relaxation of variable magnitude (Fig. 7A, B, E). d,l-Nylidrin, a synthetic β-stimulant characterized by a high degree of β-specificity, direct mechanism of action, and long duration

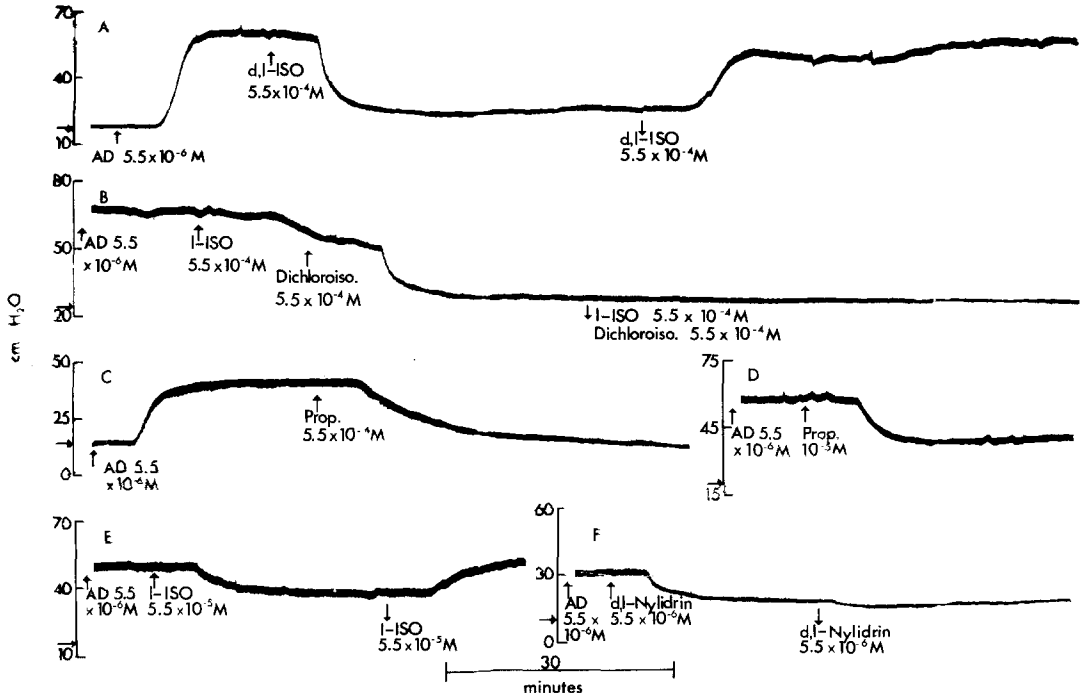

Fig. 7. Examples of the dilatory effects of various agents applied as constant concentrations in the perfusate on the tone created by 5.5×10^{-6} M AD in a number of different trunk preparations. Upward arrows indicate point of addition to the perfusate, and downward arrows, point of removal. The apparent delay between an upward arrow and the start of a response largely reflects the dead space of the perfusion system. Upward arrows to the left of a record indicate addition before the section of record shown. Horizontal arrows indicate the original perfusion pressure at baseline R_s

of effect in mammals (Innes and Nickerson, 1970) produced a similar effect (threshold: 5.5×10^{-7} M) (Fig. 7F). The isoprenaline dilations were quickly and completely reversed when the agents were removed from the perfusion medium (Fig. 7A, E), but the d,l-nylidrin effect persisted for at least 90 min (Fig. 7F). Attempts to specifically block the isoprenaline effects with propranolol or dichloroisoproterenol again proved unsuccessful because of the simultaneous reduction of α-adrenergic tone. At 5.5×10^{-4} M in the perfusate, both β-antagonists virtually abolished the constriction caused by 5.5×10^{-6} M AD (Fig. 7B, C).

The anti-α-adrenergic influence of the β-blockers could indicate a lack of adrenergic receptor specificity (with respect to antagonist action) in the trout, and thus a difference from higher vertebrates. The nature of this phenomenon was therefore further investigated on the injection dose/response curve to AD. Both propranolol and dichloroisoproterenol at 5.5×10^{-4} M in the perfusate completely abolished the AD response up to a dose of 1 μmole (Fig. 8A, B). Indeed, massive doses of AD (100 μmoles) were without effect (3 preparations). A much lower concentration of propranolol (3.2×10^{-5} M) depressed the re-

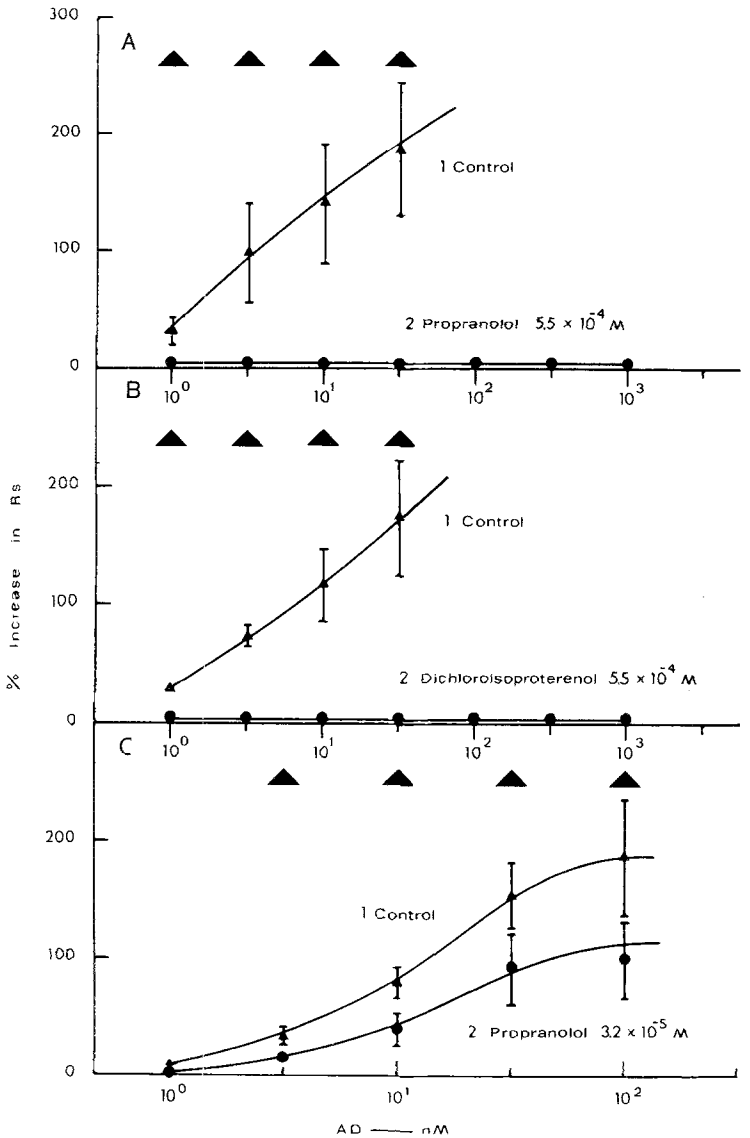

Fig. 8. The effects of the β-adrenergic antagonists (A) 5.5×10^{-4} M propranolol ($N=4$), (B) 5.5×10^{-4} M dichloroisoproterenol ($N=4$), and (C) 3.2×10^{-5} M propranolol ($n=8$) on the constrictory dose/response curve to AD. Means ± 1 S.E. Large triangle $=p<0.05$. 1, ▲ control; 2, ● after β-blocker

sponse to AD by about 45% at every dose without shifting the curve along the dose axis (Fig. 8C). In a single experiment, dichloroisoproterenol had a similar effect at 3.2×10^{-5} M. As described previously, at 10^{-5} M the two β-antagonists had no significant influence on the AD dose/response curve (Fig. 4). This pattern of inhibition, by a constant proportion, of the agonist

dose/response curve by low concentrations of the blocker, and complete suppression by high concentrations, is characteristic of non-competitive antagonism (Ariens, 1964).

In view of the close structural similarity between the β-blocking agents and isoprenaline (Moran, 1967), it seemed possible that the isoprenaline stimulated dilations during α-adrenergic tone could also reflect non-competitive α-adrenergic antagonism. However a number of different experimental approaches indicated a completely separate mechanism of action of isoprenaline from that of propranolol and dichloroisoproterenol. Firstly, isoprenaline had a qualitatively different effect on the injection dose/response curve to AD (see below; compare Fig. 10 with Fig. 8). Secondly, whereas the isoprenaline dilations were quickly and completely reversible (Fig. 7A, E), the effects of the β-blockers persisted (Fig. 7B); after 5.5×10^{-4} M dichloroisoproterenol or propranolol, only slight recovery of the α-adrenergic tone (up to 30% of control values) ever occurred. Thirdly, in experiments where R_s was increased by use of a high potassium perfusion medium, rather than by AD or NAD, isoprenaline did not mimic the effects of the β-blockers.

Elevation of the perfusate potassium concentration produced a vasoconstriction roughly proportional to the hyperkalaemia (threshold $= 2 \times 10^{-2}$ M) and characterized by a large initial overshoot followed by stabilization (Fig. 9). At 10^{-1} M potassium, the stable constriction was 217.8% ± 12.6% (4) of baseline R_s. d,l-ISO and l-ISO (5.5×10^{-4} M in the perfusate) did not affect this potassium dependent tone (Fig. 9), but both propranolol (Fig. 9) and dichloroisoproterenol at 5.5×10^{-4} M produced marked relaxations. As high potassium solutions normally produce contraction by a direct depolarization of the muscle membrane (Peiper et al., 1972), these observations indicate a direct, perhaps

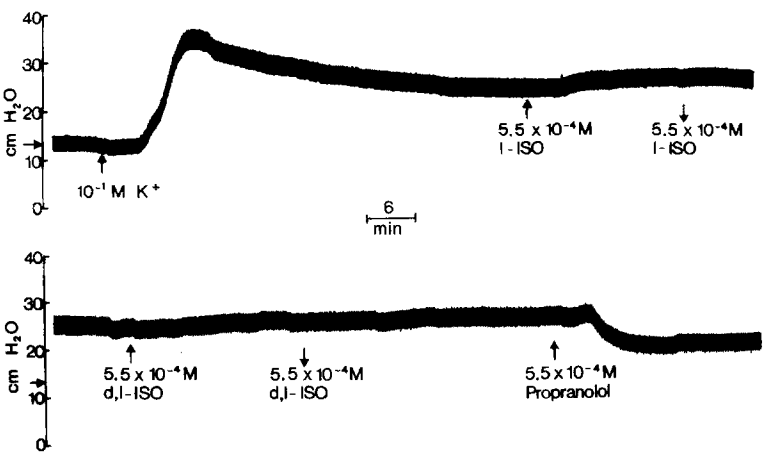

Fig. 9. Typical constrictory effect of a 10^{-1} M potassium perfusion medium, and the actions of 5.5×10^{-4} M l-ISO, 5.5×10^{-4} M d,l-ISO, and 5.5×10^{-4} M propranolol on this potassium dependent tone in a single preparation. The two parts of the record are in fact continuous. Symbols as in Figure 7. Note the lack of effect of l-ISO and d,l-ISO but pronounced dilation by propranolol at equal concentrations

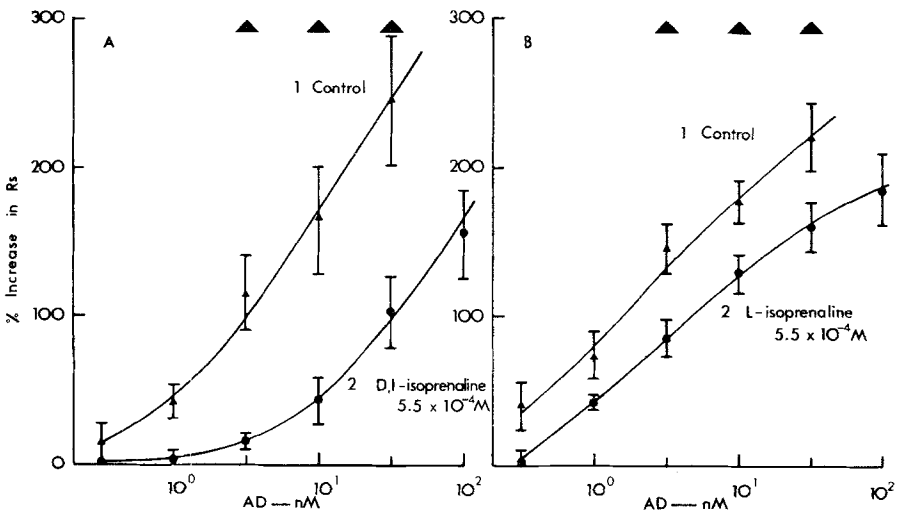

Fig. 10. The effects of (A) 5.5×10^{-4} M d,l-ISO ($n=7$) and (B) 5.5×10^{-4} M l-ISO ($n=6$) on the constrictory dose/response curve to AD. Means ± 1 S.E. Large triangle $= p < 0.05$. 1, ▲ control; 2, ● after isoprenaline

toxic effect of high concentrations of the β-blockers, but not of l-ISO and d,l-ISO, on the contractile tissue at a point distal to the adrenergic receptors.

The racemic mixture d,l-ISO seemed more potent than the pure isomer l-ISO in causing dilation from the tone of 5.5×10^{-6} M AD, and this difference was confirmed in 4 experiments in which equal concentrations (5.5×10^{-4} M) of the two compounds were compared. Although the absolute sizes of the dilations varied widely between preparations, the ratio (l-ISO/d,l-ISO) of their effects was quite uniform (mean=0.36; range=0.35–0.39).

The problem was further studied by examining the actions of the two substances on the injection dose/response curve to AD. Both d,l-ISO and l-ISO (5.5×10^{-4} M in the perfusate) produced approximately parallel shifts to the right of the relationship, and the former was again more potent (Fig. 10A, B). The ratio (l-ISO/d,l-ISO) of the depressant effects of the two compounds over most of the curves was about 0.37, in close agreement with the value (0.36) found in the constant concentration experiments. As equimolar concentrations of the two substances were used, their "α-blocking" potencies could be evaluated by comparison of the dose ratios—i.e. the ratio, over the linear part of the curve, of the dose of AD needed to produce equal responses before and after the application of isoprenaline. The dose ratios were 4.3 for l-ISO and 13.7 for d,l-ISO; thus the racemate was about 3.2 times as potent as the l-isomer. As the interpretation of these results is complex (see Discussion), a completely different experimental approach was adopted to gain additional evidence on the presence or absence of β-receptors.

On both the α- and β_2-adrenergic receptors of higher vertebrates, AD is more potent than NAD (Ahlquist, 1948; Arnold, 1972), but NAD has a much greater α/β_2 ratio of stimulating activity (Furchgott, 1967; Innes and Nickerson,

1970). Moreover NAD is more potent than AD on receptors of the β_1-variety (Arnold, 1972). If the net constriction caused by a dual agonist such as AD or NAD in the trunk of the trout is a "compromise" between α-contraction and β-relaxation, then the AD/NAD potency ratio for vasoconstriction should increase after β-blockade if β_2-receptors are present, and should decrease if β_1-receptors are present. However if no β-receptors are present, the ratio should remain unchanged. These effects should be independent of any non-competitive α-adrenergic antagonism exerted by the β-blocker (e.g. Fig. 8). Thus AD/NAD potency ratios were measured in the perfused trunk preparation before and after treatment with propranolol (10^{-5} M in the perfusate). Injection dose/response curves to AD and NAD were compared in the response region between 10 and 32 nmoles AD. To correct for possible time dependent alterations in sensitivity, the NAD relationship was temporally bracketed by AD curves both before and after propranolol. Potencies were computed from the ratio of doses producing equal responses (Furchgott, 1967). In all of these 6 experiments, the AD/NAD potency ratio increased (from 2.31 ± 0.21 (S.E.) to 3.21 ± 0.34) ($p < 0.02$ by paired Student's t-test) after treatment with the β-antagonist, indicating the presence of β_2-dilatory adrenoreceptors in the preparation.

III. The Effects of Adrenergic Antagonists on Vascular Resistance

With the exception of dichloroisoproterenol, none of the real or suspected adrenergic blocking agents used in the study (phenoxybenzamine, 10^{-5} M; yohimbine, 10^{-5} M; propranolol, 10^{-5} M, 3.2×10^{-5} M, 5.5×10^{-4} M; d,1-ISO, 5.5×10^{-4} M; and 1-ISO, 5.5×10^{-4}) significantly altered baseline R_s ($p > 0.05$). Guanethidine (10^{-5} M), a drug which produces adrenergic neuron blockade in mammals (Nickerson, 1970), was also without effect. Dichloroisoproterenol increased baseline R_s by approximately 10% at both 10^{-5} M and 5.5×10^{-4} M, but the effect was statistically significant ($p < 0.02$) only at the lower concentration. The cause of this slight constriction by dichloroisoproterenol is unknown, but as propranolol did not duplicate the effect, a direct antagonism of a latent "β-dilatory tone" seems unlikely. Dichloroisoproterenol manifests several non-specific properties in mammals (Moran, 1967; Nickerson, 1970). Taken as a whole, these observations indicate a complete lack of adrenergic vasomotor tone in the systemic vasculature at baseline R_s.

Discussion

The present results indicate that the α-adrenergic receptors in the systemic circulation of the trout are similar to those of mammals in their AD/NAD potency ratio (3.2/1.0) (Furchgott, 1967, 1970; Wood and Shelton, 1975), the natures of their blockade by phenoxybenzamine, yohimbine, and apparently d-isoprenaline (see below), and their resistance to competitive antagonism by propranolol and dichloroisoproterenol. However the trout α-receptors differ from their mam-

malian counterparts in their insensitivity to l-ISO, PHE, d,l-methoxamine, and dopamine (see below). Only the endogenously occurring catecholamines AD and NAD are effective. The systemic α-adrenoreceptors of *S. gairdneri* therefore seem to be intolerant of deviation from the natural catecholamine structure.

Only one concentration (10^{-5} M) of yohimbine and phenoxybenzamine was employed in the present experiments (Fig. 3). Absolute proof that yohimbine and phenoxybenzamine are acting by competitive and non-equilibrium mechanisms respectively as on mammalian α-receptors demands the use of several different blocker concentrations (cf. Ariens, 1964). Nevertheless, such proof has recently been reported for the coeliac arteries of the cod (Holmgren and Nilsson, 1974). The lack of constrictory effect of l-ISO, even after β-blockade, agrees with the observations of Holmgren and Nilsson (1974). However Nilsson's laboratory found PHE to be at least a partial α-agonist in coeliac artery strips of cod and trout, as well as in the perfused swimbladder vasculature of cod and in the intact cod (Nilsson, 1972; Helgason and Nilsson, 1973; Holmgren and Nilsson, 1974). PHE had no definite pressor effect in the intact trout (Wood and Shelton, unpublished results). The cause of this disagreement is unknown.

The α-adrenergic response was quite susceptible to inhibition by high concentrations of the competitive β-blockers propranolol and dichloroisoproterenol (Figs. 7B, C, D, 8). This finding agrees with the observations of Burnstock and Kirby (1968) and Kirby and Burnstock (1969) on arterial strips from a range of lower vertebrates, and of Holmgren and Nilsson (1974) on the cod coeliac artery. In the latter preparation, the blockade was of the competitive type, while in mammals, very high concentrations of propranolol produce a mixed competitive and non-competitive α-adrenergic antagonism (Kohli and Ling, 1967). In the present study, the blockade appeared to be non-competitive (Fig. 8) with a point of action beyond the adrenergic receptor (Fig. 9). There is, therefore, no reason to believe that the trout α-adrenergic receptors themselves are less specific, with respect to antagonist action, than those of higher vertebrates.

It has previously been argued that autonomic innervation, rather than circulating catecholamine levels, provides the effective adrenergic regulation of systemic vasomotor tone in *S. gairdneri* (Wood and Shelton, 1975). The present demonstration of a slow α-adrenergic constriction caused by the indirectly acting sympathomimetic tyramine provides further evidence of a functional control of R_s by adrenergic nerves. Tyramine normally acts by liberating NAD stored in the terminals of post-ganglionic sympathetic fibres, although a similar indirect effect on vascular chromaffin tissue cannot be completely ruled out (Trendelenburg, 1963). Dopamine, which is structurally very similar to tyramine, had an apparently parallel mode of action; the crossed-tachyphylaxis between dopamine and tyramine (Fig. 5) is especially persuasive of this point (Ariens, 1964). An immediate, direct α-stimulating action of dopamine, as seen in mammals (Goldberg, 1972), did not occur in the trout. Specific dopaminergic and β-adrenergic dilatory effects of dopamine were also not observed, but dopamine was only administered at baseline R_s, where such responses might not have been detected.

Both 1-ISO and d,1-ISO at 5.5×10^{-4} M shifted the AD dose/response curve to the right in a parallel fashion, but the latter was considerably more potent in this regard (Fig. 10). Competitive antagonism (α-receptor blockade) and/or functional antagonism (β-receptor stimulation) by isoprenaline may be involved in these effects (Ariens, 1964). As d-ISO is reported to have negligible potency on vascular β-receptors (Innes and Nickerson, 1970), one would expect the racemate to be less (0.5 times) rather than more (3.2 times) potent than the 1-isomer if only β-receptor stimulation were taking place. It appears more probable that d-ISO is acting as a competitive α-antagonist, while 1-ISO is acting as a direct β-agonist. This conclusion is supported by the report that in mammals, d-ISO is in fact a competitive α-adrenergic antagonist, while 1-ISO has no α-blocking activity but strong β-stimulating activity (Ariens, 1964). In terms of net dilatory effect by the two mechanisms, d-ISO must be considerably more potent than 1-ISO, and thus the mixture d,1-ISO also more potent than 1-ISO.

Although an unequivocal demonstration of the presence of β-receptors in the trout trunk was prevented by a number of complicating properties of the system, considerable evidence in favour of their existence was obtained. The dilatory actions of 1-ISO and d,1-nylidrin during AD-mediated constriction (Figs. 6A, 7B, E, F), the parallel shift in the AD dose/response curve caused by 1-ISO (Fig. 10B), and, most importantly, the increased AD/NAD potency ratio after propranolol all support the presence of a β-dilatory mechanism. The latter result indicates that these receptors, as in the peripheral vasculature of mammals (Arnold, 1972), are of the β_2-variety. Therefore a similar evolutionary homology would seem to exist between the receptors (β_2) of teleost and mammalian systemic vessels as between the receptors (β_1) of teleost branchial vessels and mammalian coronaries (Wood, 1974a, 1975).

The relatively high dose threshold (10 nmoles), poor dose dependency, and occasional ineffectiveness of 1-ISO in causing vasodilation from the α-adrenergic tone of 5.5×10^{-6} M AD (Fig. 6) may well have been the consequences of using a dual α- and β-agonist as the constricting agent. Most (or sometimes all) of the β-dilatory receptors may already have been occupied by AD, so 1-ISO could cause only moderate further vasodilation (or none at all) at the relatively flat upper end of the β dose/response curve. A high apparent threshold and poor dose dependency would result.

All previous workers on systemic β-receptors in fish (Chan, 1967; Johansen and Reite, 1968; Schwartz and Borzelleca, 1969; Stray-Pedersen, 1970; Nilsson, 1972; Opdyke et al., 1972; Helgason and Nilsson, 1973; Holmgren and Nilsson, 1974) have apparently used d,1-ISO rather than 1-ISO as the selective β-agonist, and have ignored the possible α-blocking activity of the d-isomer in the racemate (Ariens, 1964). The present results indicate that the racemic mixture is over 3 times as potent as the 1-isomer in causing vasodilation from α-adrenergic tone, due to the competitive α-blocking activity of the d-isomer. Thus about 5/6 of the dilatory activity of d,1-ISO on the perfused trunk of the trout can tentatively be attributed to α-blockade and only about 1/6 to β-dilation. If a similar situation applies in other fish species and there is significant α-adrenergic tone in vivo as there is in *S. gairdneri* (Wood and Shelton, 1975, and unpub-

lished results), this conclusion must cast rather serious doubts on the results of all earlier investigations. It is interesting to note that Holmgren and Nilsson (1974) have also recently shown an apparently competitive α-blockade by d,l-ISO in the cod coeliac artery strip, though they did not specifically relate it to the presence of the d-isomer.

In summary, there exists a dominant α-adrenergic constrictory mechanism, and, most probably, a β-adrenergic dilatory mechanism in the systemic vasculature of the rainbow trout. The α-receptors appear similar to but more selective than those of mammals, while the β-receptors seem to be of the β_2-variety as in the homologous peripheral vasculature of higher forms.

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