A pharmacological analysis of the adrenergic and cholinergic mechanisms regulating branchial vascular resistance in the rainbow trout (*Salmo gairdneri*)

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The adrenergic and cholinergic mechanisms regulating branchial vascular resistance in the trout have been studied using a whole gill preparation perfused at constant flow under approximately normal afferent and efferent levels of blood pressure. The receptors have been pharmacologically characterized by agonist potency comparisons within individual preparations and by the use of specific antagonists. The predominant response to adrenergic stimulation is vasodilation mediated by β-receptors, but a more rapid vasoconstriction mediated by α-receptors may also occur. The β-adrenoreceptors appear to be of the β_1 variety, as in the homologous coronary vasculature of mammals. The cholinergic receptors of the gills are purely muscarinic in nature and mediate vasoconstriction. The possible functions of the mechanisms are discussed.

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On a étudié les mécanismes adrénergique et cholinergique qui controlent la résistance vasculaire branchiale chez la truite, par l'examen d'une préparation de branchie entière soumise à une perfusion à écoulement constant, les pressions de sang afférent et de sang efférent étant à peu près normales. On a déterminé les caractéristiques pharmacologiques des récepteurs par des comparaisons de potentiels agonistiques au sein des préparations individuelles et par l'utilisation d'antagonistes spécifiques. Une stimulation adrénergique cause presque toujours une vasodilatation par l'intermédiaire de récepteurs β, mais peut aussi causer une vasoconstriction plus rapide par l'intermédiaire de récepteurs α. Les adrénorécepteurs β semblent appartenir à la variété β_1, comme dans le système vasculaire coronaire homologue des mammifères. Les récepteurs cholinergiques des branchies sont de nature purement muscarinique et entraînent la vasoconstriction. On discute des fonctions possibles de ces mécanismes.

[Traduit par le journal]

Introduction

A perfused whole gill preparation has recently been developed for the study of branchial vascular resistance (*Rg*) in the rainbow trout, *Salmo gairdneri* (Wood 1974). In that investigation, the influence on *Rg* of changes in blood pressure and adrenergic stimulation were examined. The aim of the present work was to extend the original observations on the latter factor with a more detailed pharmacological analysis of the branchial adrenergic receptors. Considerable disagreement exists in the literature over the nature of these receptors in teleosts (Ostlund and Fange 1962; Chan 1967; Randall and Stevens 1967; Reite 1969; Rankin and Maetz 1971; Belaud *et al.* 1971; Randall *et al.* 1972; Bergman *et al.* 1974). In the present study, the receptors have been characterized both by agonist potency comparisons (Ahlquist 1948) within individual preparations and by examining the effects of specific antagonists on agonist dose/response curves (Ariens 1964). Some data on the cholinergic receptor system of the trout gill have also been included.

In view of the demonstrated reliability of measuring vasomotor tone changes in the gills by comparing vascular resistances (*Rg*) at the same flow ($\dot{Q}$) (Wood 1974), a constant $\dot{Q}$ perfusion technique has been adopted in the present study. This method has previously proved very suitable for pharmacological studies of the systemic vascular bed of *S. gairdneri* (Wood and Shelton 1975; Wood, in preparation). Because of the marked dependence of *Rg* on afferent (*Pg*) and efferent (*Pe*) pressures

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(Wood 1974), approximately normal in vivo values of *Pg* and *Pe* have been employed in the present experiments.

Materials and Methods

Rainbow trout (150–525 g) were acquired, maintained, and acclimated to 14.5 ± 1.5 °C as described previously (Wood 1974). Experiments were performed at 5 ± 1 °C on 18 pump-perfused gills, of which 13 provided useable results (see Results). All pressures have been expressed in units of cm of water relative to the water surface in the outer perfusion chamber as zero (see Wood 1974).

Preparation

Whole gills were prepared for experimentation using the same surgical procedures, perfusion chambers, and perfusion medium as described previously (Wood 1974), but pump perfusion at constant $\dot{Q}$, rather than vertical tube perfusion, was employed in the present study. Before use, the medium was passed through a 0.22-μ (micron) Millipore filter (Rankin and Maetz 1971). The pump perfusion equipment has been described in detail by Wood and Shelton (1975). In brief, the apparatus comprised a pressure-independent peristaltic pump (Quickfit) drawing perfusate at constant $\dot{Q}$ from a reservoir resting on a top-loading balance (for measurement of $\dot{Q}$) and impelling it through a flask containing an air bubble for depulsation. The flow then passed directly into the ventral aorta of the preparation; perfusion pressure was monitored with a Sanborn 267 BC pressure transducer through a T-joint between the depulsator and the point of cannulation. The preparation itself was fixed in a Perspex chamber which could be filled with saline to apply a constant *Pe* to the dorsal aorta while allowing external irrigation of the gills with aerated freshwater (see Fig. 1 of Wood 1974).

In all runs, branchial water flow was set at 100 ± 20 ml/100 g body weight per min, and *Pe* at 40 cm H_2O to approximate the normal in vivo dorsal aortic blood pressure. Constant perfusion rates between 0.25 and 0.75 ml/100 g per min were used in different experiments, yielding values of *Pg* between 45 and 75 cm H_2O, which approximates the physiological range of ventral aortic pressure in the trout. Because of the effectively large resistance offered to the pulsatile pump output by the gill preparation plus the *Pe* of 40 cm H_2O, it was necessary to use a relatively large air bubble (approximately 10 ml) in the flask for adequate depulsation (pulse pressure = 4–8 cm H_2O). This introduced a latency (response time = 8–15 s) into the system which unfortunately tended to obscure some rapid responses (see Results). At the end of an experiment, the pressure drop across the perfusion catheter distal to the point of pressure measurement was determined, and changes in *Rg* calculated as described by Wood and Shelton (1975).

Drugs

(i) Agonists: 1-adrenaline bitartrate (AD), 1-noradrenaline bitartrate (NAD), 1-isoprenaline bitartrate (ISO), 1-phenylephrine hydrochloride (PHE), and acetylcholine chloride (ACH) (all Sigma).

(ii) Antagonists: yohimbine hydrochloride, propranolol hydrochloride, dichloroisoproterenol hydrochloride, atropine sulfate (all Sigma), and *d*-tubocurarine chloride (Burroughs Wellcome).

Agonists were administered as discrete doses by injection (volume = 0.1 ml) through a sidearm into the perfusion line immediately proximal to the point of cannulation. A momentary spike on the perfusion pressure record marked the time of injection. Dose/response studies were carried out with a logarithmically increasing dose order. Antagonists were added directly to the perfusion reservoir and allowed to act for at least 30 min (usually 60 min) 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 every 3 h.

Treatment of Data

All responses by the gill to drug administration have been expressed as the maximum percentage change of the value of *Rg* immediately preceding injection (at the same $\dot{Q}$). The rate of spontaneous *Rg* alteration was much lower than in the vertical tube work (Wood 1974), so no correction has been made for this factor. Because of the limited number of animals studied, all data have been expressed as the results of individual experiments. However all experiments were replicated at least 3 times on different preparations with similar results, and no contradictory data were obtained, unless otherwise stated.

Results

Characteristics of the Preparation

The initial *Rg* of the pump perfused gill at $\dot{Q}$ = 0.46 ± 0.03 (13) ml/100 g body weight per min (mean ± 1 SE (*N*)) was 26.30 ± 3.20 (13) cm $H_2O \cdot min \cdot 100$ g/ml, a value close to that (20.86 ± 1.16 (54) cm $H_2O \cdot min \cdot 100$ g/ml) initially recorded at a comparable flow (0.50 ml/100 g per min) with the vertical tube technique (Wood 1974). *Rg* increased significantly ($p < 0.001$) at a variable rate over the next 45 min to 4 h, but in all cases eventually reached a stable level about twice as large as the original (49.21 ± 5.51 (13) cm $H_2O \cdot min \cdot 100$ g/ml). As *Rg* increased, adrenergic dilatory responses (see below) rose in terms of the absolute change in *Rg* for a certain dose, but remained remarkably stable in terms of the percentage change in *Rg*. This would tend to indicate that a real increase in vasomotor tone rather than a mechanical problem such as oedema or particulate blockage was responsible. In any event, previous work has indicated that these mechanical factors are of minimal influence (Wood 1974). However, none of the adrenergic or cholinergic blocking agents employed in the study prevented the phenomenon, so its mechanism remains elusive.

Spontaneous alterations in sensitivity to

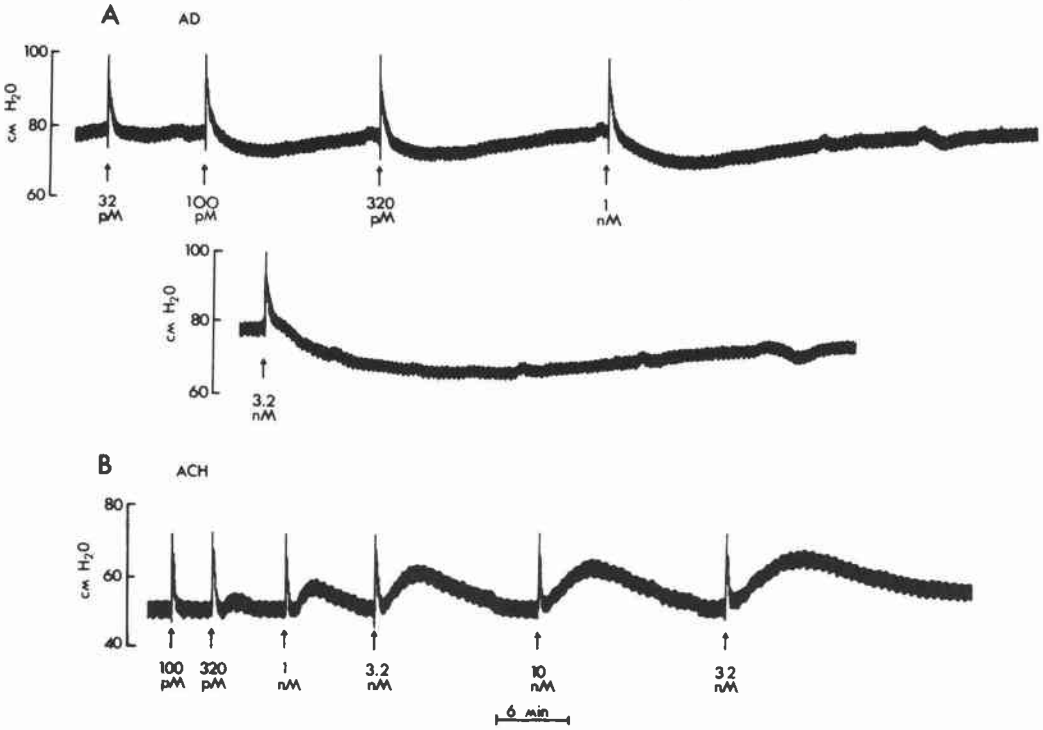

FIG. 1. A. Typical perfusion pressure record of a dilatory dose/response curve to AD. A slight constrictory effect may have been masked by the injection artifacts. The response to 32 p*M* AD was essentially similar to the response to the saline vehicle (not shown) and therefore subthreshold. $\dot{Q}$ = 0.57 ml/100 g per min. Pressure drop across perfusion cannula = 9 cm H_2O; *Pe* = 40 cm H_2O. B. Typical perfusion pressure record of a constrictory dose/response curve to ACH. $\dot{Q}$ = 0.25 ml/100 g per min. Pressure drop across perfusion cannula = 6 cm H_2O; *Pe* = 40 cm H_2O.

adrenergic agonists caused some difficulty in the present preparation. In a few cases, gradual elevations of sensitivity took place, but in most, an abrupt loss of sensitivity occurred without forewarning. The five gills rejected because of their low sensitivity right from the start of the experiments may have been subject to a similar phenomenon. No explanation can be given for the problem; it did not seem to be time-related (indeed two preparations maintained good reactivity for 14 h) and oxygenation of the perfusate, which was of importance in the trunk (Wood and Shelton 1975), did not appear to have a salutary influence.

Adrenergic Studies

In mammals, ISO is relatively pure β-adrenergic stimulant, while the other two true catecholamines AD and NAD are dual stimulants, acting on both α- and β-adrenergic receptors. When administered to the trout gill, all three amines caused dose-dependent vasodilation. The response was relatively slow, taking at least 2 min to reach a maximum effect, and at high doses up to 15 min (Fig. 1A). Sensitivities varied somewhat between gills, but the branchial vasculature was obviously much more sensitive to injected catecholamines than was the systemic vasculature. Representative threshold doses were 3.2 p*M* for ISO and 32 p*M* for AD and NAD in the gill versus 320 p*M* to 1 n*M* for AD and NAD in the trunk (Wood, in preparation). Maximum dilatory responses of the gill to the three catecholamines were of similar magnitude (dose: ISO, approximately 10 n*M*; AD and NAD, approximately 100 n*M*), averaging a 59.6 ± 4.1% (8) decrease in *Rg*. This value is in close agreement with the figure of 60% found in the vertical tube perfusions (Wood 1974).

The results of potency comparisons of the three agonists for vasodilation within individual preparations (determined by the method of

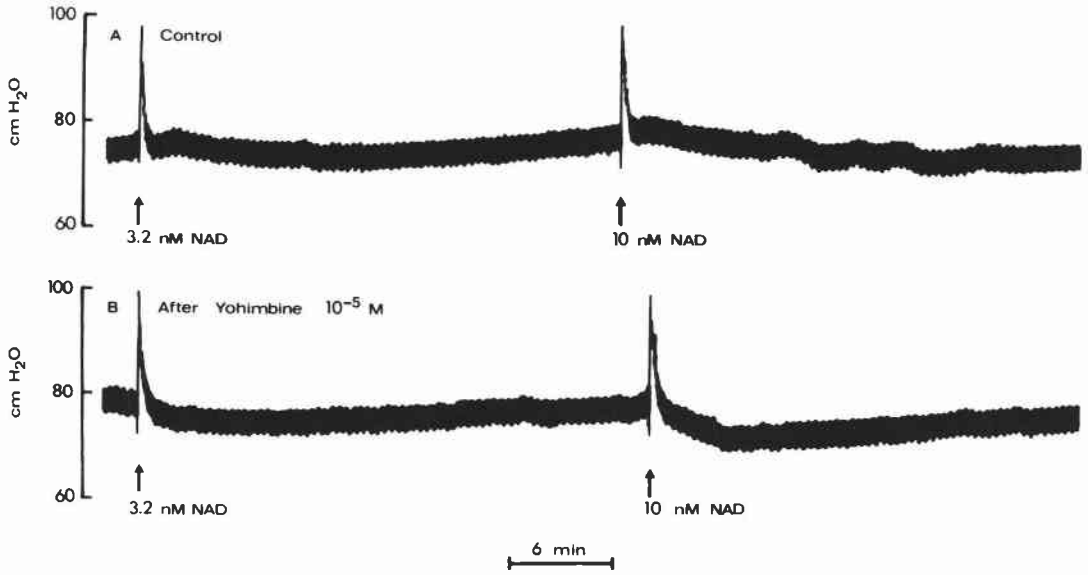

FIG. 2. Perfusion pressure records showing the effects of the α-adrenergic antagonist yohimbine (10^{-5} *M*) on the dual responses to NAD. $\dot{Q}$ = 0.71 ml/100 g per min. Pressure drop across perfusion cannula = 10 cm H_2O; *Pe* = 40 cm H_2O. A. Control. Note the small initial pressor and following depressor responses. B. After 10^{-5} *M* yohimbine. Note that the pressor responses were eliminated but the depressor responses remained unaffected.

TABLE 1

Vasodilatory potency ratios between ISO, NAD, and AD within individual gill preparations

Experiment	ISO	NAD	AD
1	18.6	—	1.0
2	9.3	—	1.0
3	—	2.7	1.0
4	25.0	4.7	1.0
$\bar{x}$	17.6	3.7	1.0

NOTE: As AD was used in all four experiments, its potency has been set to 1.0.

Furchgott (1967)) are presented in Table 1. Although variable and limited in number, these data confirm the potency order ISO > NAD > AD previously found by comparison of mean concentration/response curves in gills perfused by the vertical tube technique (Wood 1974).

In 5 of the 13 preparations examined, AD and NAD caused definite small constrictions (5–25% increases in *Rg*) preceding the dilatory responses (Fig. 2). ISO never had this effect. The pressor responses showed little dose dependency, probably because they were reversed by the overriding dilatory responses before reaching maxima. Constrictions were seen most distinctly with small doses of AD and NAD and when *Rg* was lowest at the start of perfusion, i.e. in situations where the absolute dilatory effects of the catecholamines were minimal. In fish in which these excitatory responses were not clearly seen, there was often a slight thickening of the pressure trace following the injection peak (Figs. 1A, 3). This phenomenon probably represented a real pressor effect obscured by the slow response time of the recording system (see Materials and Methods) and (or) the overshadowing dilation. In cases where the latter influence was dominant, selective blockade of the relaxation (see below) clearly revealed the constriction (Fig. 3).

The specific β-adrenergic antagonists dichloroisoproterenol and propranolol at 10^{-5} *M* both produced a typical competitive blockade of the dilatory responses to AD, NAD, and ISO, shifting the dose/response curves to the right in a parallel fashion (Figs. 3, 4). The dose ratio range (i.e. the distance on the logarithmic dose axis of the shift) was similar for the different agonists (e.g. Fig. 4), indicating that the three catecholamines were all acting on the same β-receptor system (Ariens 1964). Although antagonist potencies were not systematically compared, propranolol appeared to be a more potent blocker than dichloroisoproterenol, which is in

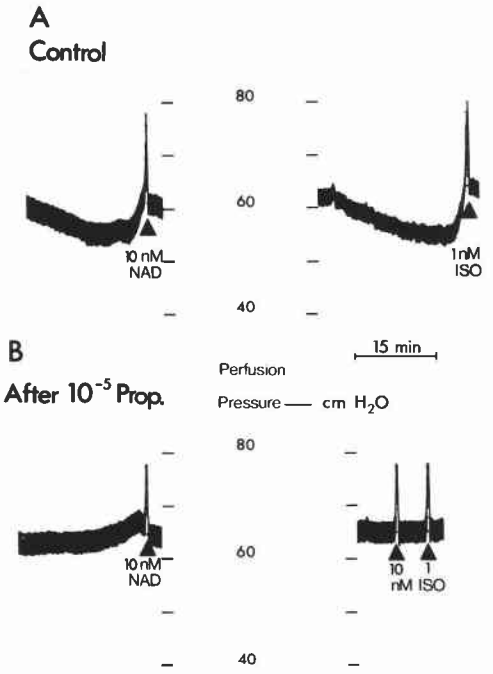

FIG. 3. Perfusion pressure records showing the effects of the β-adrenergic antagonist propranolol (10^{-5} *M*) on the responses to NAD and ISO. Note that time proceeds from right to left in these traces. $\dot{Q}$ = 0.39 ml/100 g per min. Pressure drop across perfusion cannula = 11 cm H_2O; *Pe* = 40 cm H_2O. A. Control. Note the approximately equal dilatory effects of 10 n*M* NAD and 1 n*M* ISO, and the slight thickening at the base of the injection artifact (to NAD only) which represents a small pressor response unmasked in B. B. After 10^{-5} *M* propranolol. Note that the depressor responses to NAD and ISO were eliminated but that a definite pressor response to NAD, but not to ISO, was revealed.

agreement with findings on mammalian β-receptors (Moran 1967).

In the short term (30–60 min), propranolol and dichloroisoproterenol at 10^{-5} *M* either did not affect or slightly enhanced the rapid pressor responses to AD and NAD, and in some cases exposed a definite vasoconstriction which had previously been masked by vasodilation (Fig. 3). ISO remained without constrictory influence (Fig. 3). As application of the β-blocking agents was continued, the constrictions become reduced, and, in one experiment, disappeared completely after 2 h of treatment with 10^{-5} *M* dichloroisoproterenol. Nevertheless, the pressor effects were generally much more resistant to the β-antagonists than were the depressor effects. Because of the gradual reduction of the constrictory responses by the β-blocking agents, it was unfortunately not possible to make accurate potency comparisons of the pressor agonists during β-blockade. However single-dose comparisons of AD and NAD in the presence of 10^{-5} *M* propranolol (two experiments) indicated that the two amines were approximately equiactive.

The selective α-adrenergic antagonist yohimbine (10^{-5} *M*) abolished the pressor responses at all levels of AD and NAD tested (up to 320 n*M*) (Fig. 2). The magnitude of the depressor responses to these agents remained virtually unchanged, though they occurred more rapidly than before due to the elimination of the initial constriction (Fig. 2). The vasodilation caused by ISO was unaffected by yohimbine (10^{-5} *M*).

PHE, which is a selective α-adrenergic agonist in mammals, had no constrictory effect in the trout gill, but caused small and erratic dilatory responses (5–25% decreases in *Rg*) over the dose range 100 p*M* to 1 μ*M*. The magnitude of the dilations showed virtually no dose dependency. These results are in agreement with previous findings of an anomalous action of PHE on both the branchial (Wood 1974) and systemic vascular beds (Wood and Shelton 1975) of the trout.

Cholinergic Studies

ACH (threshold = 100 p*M*) caused dose-dependent vasoconstriction in the perfused gill preparation (Fig. 1B). These constrictory responses generally developed and decayed more rapidly than the dilatory responses to catecholamines (Fig. 1). The maximum cholinergic response was not determined, but ACH was obviously an extremely effective vasoconstrictor, causing increases in *Rg* of 250–500% at 10 n*M* (Figs. 1B, 5).

In higher vertebrates, ACH can stimulate both nicotinic and muscarinic cholinoreceptors. The selective nicotinic antagonist *d*-tubocurarine (10^{-5} *M*), which is effective in blocking nicotinic receptors elsewhere in the trout vascular system at this concentration (Wood, unpublished results), did not inhibit the branchial effects of ACH. Indeed, there seemed to be a slight potentiation of the responses (Fig. 5). However, the muscarinic antagonist atropine at 10^{-7} *M* exerted a classical competitive blockade of the pressor action of ACH in the gills (Fig. 5). (The blockade was similar whether or not *d*-tubocurarine (10^{-5} *M*) had been previously

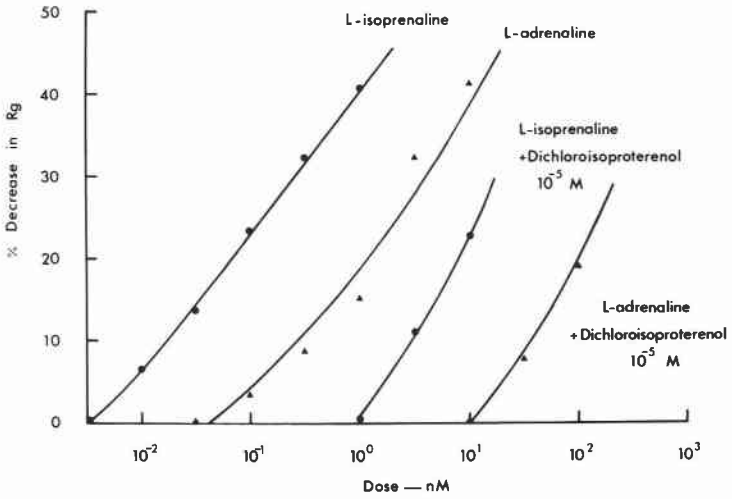

FIG. 4. Results of a typical experiment showing the effects of the competitive β-adrenergic antagonist dichloroisoproterenol (10^{-5} *M*) on the dilatory dose/response curves to ISO (●) and AD (▲). Note the approximately parallel displacement to the right of the curves and approximately equal shifts of the ISO and AD relationships.

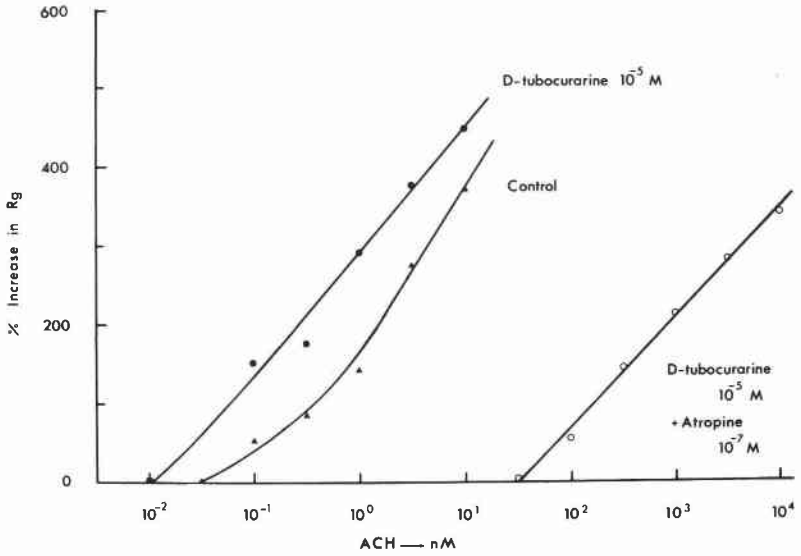

FIG. 5. Results of a typical experiment showing the effects of the nicotinic cholinergic antagonist *d*-tubocurarine (10^{-5} *M*) and the muscarinic cholinergic antagonist atropine (10^{-7} *M*) on the constrictory dose/response curve to ACH. Note the slight potentiation of the ACH dose/response curve by *d*-tubocurarine, and the approximately parallel displacement to the right of the relationship by atropine.

applied.) At 10^{-6} *M*, atropine shifted the ACH threshold to 320 n*M*, but even at this high dose level of the agonist, there was no evidence of a nicotinic effect which might previously have been masked by muscarinic constriction. No dilatory responses to ACH were observed.

Discussion

The present results with selective adrenergic blocking agents and agonist potency comparisons within individual preparations have shown that the adrenoreceptors mediating vasodilation in the trout gill are β-adrenergic in nature. The

TABLE 2

A comparison of the vasodilatory potency ratios of ISO, NAD, and AD in the trout gill with the values reported for β_1- and β_2-adrenergic receptor types in mammals

Receptor type	ISO	NAD	AD	Reference
Trout gill	17.6	3.7	1.0	Present study
β_1, mammal	10–40	1–10	1.0	Bohr (1967); Arnold (1972)
β_2, mammal	2–10	<0.05	1.0	Furchgott (1967); Arnold (1972)

actual agonist potency ratios for dilation within individual gills fall well within the ranges reported for β_1-receptors in mammals and outside the ranges reported for β_2-receptors (Table 2). The presence of β_1-receptors in the branchial vasculature of the trout had previously been suggested on the basis of the same potency order (ISO > NAD > AD) as found here, but calculated from mean agonist dose/response curves from separate groups of animals (Wood 1974). The present results (Table 1) indicate, however, that the potency of ISO relative to NAD and AD is considerably less than the earlier estimate (ISO : NAD : AD, 100:3.3:1) of Wood (1974). It is interesting to note that β_1-adrenergic receptors also apparently occur in the mammalian coronary vasculature (Bohr 1967; Arnold 1972), which bears a homologous relationship to the branchial vessels of teleosts (Keys and Bateman 1932).

Adrenergic stimulation of the gills also often caused a relatively rapid vasoconstriction preceding the β-vasodilation. Again, as in mammalian coronaries (Zuberbuhler and Bohr 1965; Bohr 1967; Trinker 1973), this mechanism appeared to be α-adrenergic in nature, being activated by AD and NAD but not by ISO. Its elimination by the α-adrenergic antagonist yohimbine and relative resistance to the β-adrenergic blockers propranolol and dichloroisoproterenol corroborated this conclusion. The eventual partial or complete inhibition of the pressor responses by the β-antagonists was similar to an anti-α-adrenergic effect of these substances in the systemic vasculature of the trout (Wood, in preparation). From that study, evidence is available that the β-blockers interfere with α-responses in a non-competitive manner with a point of action beyond the α-adrenergic receptor.

The discovery of opposing α-constrictory and β-dilatory receptors in the branchial vessels of *S. gairdneri* agrees with the recent observations of Bergman *et al.* (1974) on this species, and with the earlier findings of Reite (1969) on *Ophiodon elongatus* and *Anguilla anguilla*, and of Belaud *et al.* (1971) on *Conger conger*. Previous investigations on the salmonid gill (Randall and Stevens 1967; Richards and Fromm 1969; Randall *et al.* 1972; Wood 1974) have differed from the present conclusions in finding α- and β-dilatory receptors and (or) in failing to find α-constrictory receptors. However, in all these studies, the pharmacological characterizations performed were limited, and the techniques employed were probably incapable of detecting a rapid vasoconstrictory response in the gills.

In the past, decreases in *Rg* and increases in O_2 uptake have often been related to the shunting of branchial blood flow from "non-respiratory" to "respiratory" pathways in the gill filaments (Steen and Kruysse 1964; Richards and Fromm 1969). However, a strong body of evidence against the functional presence of the "non-respiratory" shunts has recently appeared, and it now seems that such effects can be better explained by the recruitment of more secondary lamellae in terms of both blood and water flow (Hughes 1972; Hughes and Morgan 1973; Morgan and Tovell 1973; Gannon *et al.* 1973; Vogel *et al.* 1973; Cameron 1974; Wood 1974). As yet, there exists no concrete evidence of the location of the adrenergic mechanisms regulating *Rg* or of the relative roles of the branchial α- and β-receptors in vivo. However, the homologous mammalian coronary circulation may offer some clue to the situation in the teleost gill. In the coronary vasculature of dogs, the closer to the ascending aorta that a vessel is located, the higher is its ratio of α- to β-adrenergic activity. Thus α-constriction predominates in large arteries and β-dilation in small arteries

and arterioles; the latter are more important in setting the total resistance of the coronary vascular bed (Zuberbuhler and Bohr 1965; Bohr 1967; Parrat 1969). Similarly, β-dilation is the prevailing response of the branchial vascular bed to circulating catecholamines. One may speculate that α-constriction reflects a normally nerve-mediated response located in the large gill arteries such as the afferent branchials and afferent filamentals, while β-dilation reflects a largely hormonal response at the level of the lamellar arterioles. The β-mechanism may set the general level of lamellar perfusion and vascular resistance in the gills, while the α-mechanism may mediate local variations in the ventilation/perfusion ratio at the level of the filament or gill arch.

Bergman *et al.* (1974) have recently reported that cholinergic vasoconstriction in the perfused gill arch of the trout could be almost completely blocked either by the muscarinic antagonist atropine at 10^{-7} *M* (as in the present study) or by the nicotinic antagonist hexamethonium at 5×10^{-5} *M*. This result would seem to indicate either that the cholinoreceptors involved were relatively unselective or that one of the blocking agents was acting non-specifically. In the present work, another nicotinic antagonist *d*-tubocurarine (10^{-5} *M*) did not inhibit the response to ACH in the perfused whole trout gill. Tubocurarine is more potent than hexamethonium on nicotinic receptors of both the neuromuscular and ganglionic varieties in mammals (Bowman and Webb 1972), and is effective in blocking nicotinic receptors elsewhere in the circulation of the trout (Wood, unpublished results). It therefore seems probable that the cholinoreceptors involved are wholly muscarinic in nature, and that the results of Bergman *et al.* (1974) reflect a non-specific effect of hexamethonium.

The presence of a muscarinic cholinergic mechanism for vasoconstriction in the gills of *S. gairdneri* is in accord with findings in other teleost species (Ostlund and Fange 1962; Belaud *et al.* 1971), and contrasts with the apparent lack of such a mechanism in the systemic vasculature (Wood, unpublished results). As muscarinic receptors are normally located on the vascular end-organ, and a hormonal role for ACH is most unlikely, one must suppose that the receptors involved are normally activated by innervation, probably of vagal origin (Burnstock 1969). In view of the high efficacy of ACH in causing vasoconstriction in the trout gill, neural cholinergic control may be an important determinant of *Rg* in vivo.

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