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SHORT COMMUNICATION

THE CONCENTRATION DEPENDENCE OF THE STIMULATORY EFFECTS OF CATECHOLAMINES ON THE RATE AND FORCE OF CONTRACTION OF THE HEART OF DOGFISH (*SCYLIORHINUS CANICULA*)

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An adrenergic control of the elasmobranch heart has been inferred from pharmacological studies. Catecholamines have been repeatedly demonstrated to stimulate the frequency and/or force of the dogfish heart (MacDonald, 1925; Lyon, 1926; Hiatt, 1942; Ostlund, 1954; Fange & Ostlund, 1954; Capra & Satchell, 1977), and such effects have been shown to be reduced by propranolol, a beta-adrenergic antagonist (Capra & Satchell, 1977). However, the mechanism and functional significance of an adrenergic control of the elasmobranch heart remain to be demonstrated.

In contrast to most other vertebrates, a significant sympathetic innervation of the heart appears to be lacking in elasmobranchs (Nilsson, 1983). Although several investigators have described communicating branches between the vagus nerve and sympathetic nervous system (Izquierdo, 1930; Young, 1933), which may account for the presence of catecholamines in the myocardium (Ostlund, 1954; von Euler & Fange, 1961; Gannon *et al.* 1972; Saetersdal *et al.* 1975; Abrahamsson, 1979), electrical stimulation of cardiac efferent fibres in atropinized dogfish fails to elicit significant increases in heart rate or stroke volume (Izquierdo, 1930; Short *et al.* 1977). In the absence of a functional sympathetic innervation, an adrenergic control of the dogfish heart, if present, must rely on a mechanism of humoral transmission, whereby adrenergic stimulation of the heart is mediated by catecholamines liberated into the circulation at a distance from the heart. Although several stimuli have been shown to increase the concentration of plasma

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catecholamines in dogfish (Butler *et al.* 1978, 1986; Opdyke *et al.* 1981, 1982), it is not known whether physiological concentrations of catecholamines in dogfish plasma are sufficient to influence the performance of the heart.

The objective of the present study was to determine the relationship between concentration and response for the *in vitro* stimulatory effects of catecholamines on the force and frequency of contraction of the heart of the dogfish, in order that the required concentrations of catecholamines could be compared with the range of concentrations known to occur in dogfish plasma *in vivo*. Concentration–response relationships were determined for the maximum developed isometric tension of paced atrial and ventricular strips (1 mm width), and for the frequency of contraction of the isolated sinus venosus and atrium, of the heart of the dogfish (*Scyliorhinus canicula*). Experiments were performed using 40 ml tissue baths containing a physiological saline solution (Capra & Satchell, 1977) aerated with 100 % oxygen and maintained at 14 ± 0·5 °C. Atrial and ventricular strips were pre-stretched to a length at which their developed tension was 80 % of maximum, and were paced with twice-threshold stimuli (5 ms) at a frequency of 10 stimuli min^{-1}. Isolated sinus venosus and atrium preparations were preloaded with sufficient tension that their spontaneous contractions deflected the attached arm of an isotonic displacement transducer by approximately 5 mm. Drug solutions were prepared 20 min before use, and were added to the tissue bath in 200 μl aliquots. Stock solutions were prepared so that each administration of drug increased its concentration in the bath three-fold, beginning at 2 nmol l^{-1}. Sequential doses were administered (without flushing) 1 min following the completion of the response to the previous drug application, or 4 min following the previous administration if a response was not observed. The drug concentration eliciting half the maximum response (EC_{50}) was determined graphically for each preparation. All calculations were performed using the negative logarithm of the drug concentration. All drugs (isoprenaline-HCl, ISO; adrenaline bitartrate, AD; noradrenaline bitartrate, NA) were obtained from Sigma.

The relationships between catecholamine concentration and stimulation of the rate and force of contraction are shown in Fig. 1, in which the responses have been expressed as percentages of their control values. The responses to ISO, AD and NA were most pronounced in paced atrial strips, in which an approximate tripling of the maximum developed tension could be elicited. The maximum increase in the peak developed tension of paced ventricular strips amounted to an approximate doubling of control values. The rate of spontaneous contraction of the sinus venosus and atrium was less responsive to stimulation by catecholamines, the maximum response consisting of a 20–40 % increase from the control rate.

To compare the potencies of ISO, AD and NA, the concentration–response relationships of Fig. 1 were normalized to the maximum response of each preparation (Fig. 2). The mean EC_{50} values for each relationship are listed in Table 1. Although the ratios of agonist potencies varied in the three preparations, the potency order was ISO ≥ AD > NA in each case (Table 1; Fig. 2). This potency order is consistent with that previously reported by Capra & Satchell

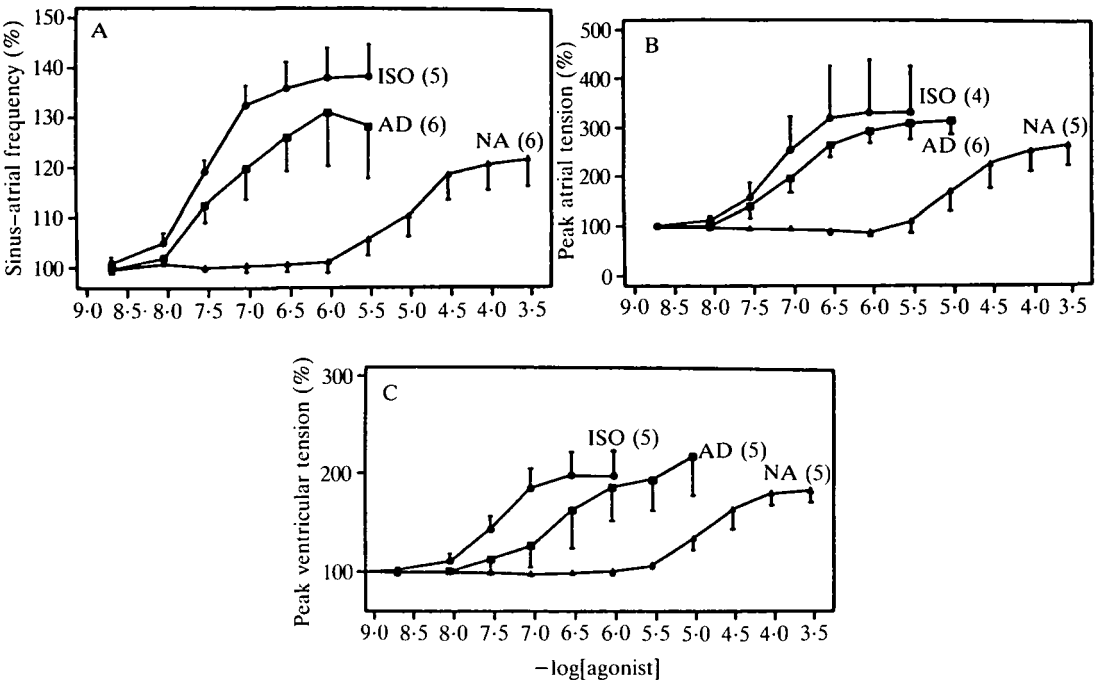

Fig. 1. Cumulative concentration–response relationships for the stimulation of dogfish myocardium by catecholamines. (A) Effect of catecholamines on the frequency of contraction of the isolated sinus venosus and atria (control frequency = 31 ± 2 beats min^{-1}). (B) Effect of catecholamines on peak developed tension of isometrically contracting paced atrial strips. (C) Effect of catecholamines on peak developed tension of isometrically contracting paced ventricular strips. All values (mean + S.E.M.) are expressed as percentages of the control value. The number of experiments used in calculating the means are given in parentheses. ISO, isoprenaline; AD, adrenaline; NA, noradrenaline.

(1977) for *Squalus acanthias* ventricle, and is also consistent with the relative protencies of AD and NA in stimulating the β_2 subgroup of adrenergic receptors, as originally defined in by Lands *et al.* (1967).

To determine whether physiological concentrations of catecholamines are sufficient to influence the dogfish heart, the range of reported dogfish plasma catecholamine concentrations has also been provided in Fig. 2 for comparison with the concentration–response relationships. The individual literature values used to determine the range of plasma catecholamine concentrations, and the conditions under which they were determined, are listed in Table 2. The range of AD concentrations which have been reported in dogfish plasma overlap with those which were found to be effective in stimulating the rate and strength of contraction of the dogfish myocardium in the present study (Tables 1, 2; Fig. 2). The lower range of plasma AD concentrations, obtained from resting dogfish (6–26 nmol l^{-1}, Table 2), represent the approximate threshold of the dogfish myocardium to

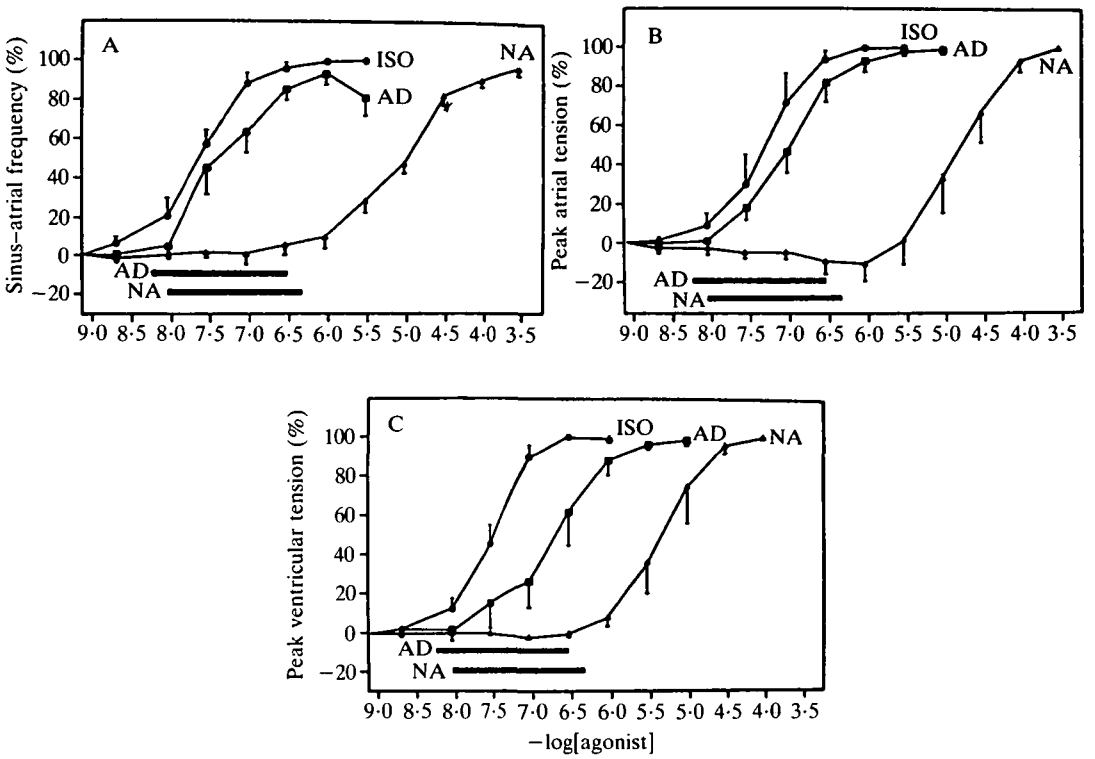

Fig. 2. Cumulative concentration–response relationships normalized to the maximum response, for the comparison of agonist potencies. All values (mean + S.E.M.) were calculated from the raw data presented in Fig. 1. Bars shown below concentration–response curves show the range of plasma catecholamine concentrations reported in living dogfish (Table 2).

catecholamines, whereas the highest reported value (284 nmol l^{-1} in acute hypoxia, Table 2) corresponds with those producing 60–90 % of the maximum response of the heart to catecholamines in the present study. In contrast, even though plasma NA concentrations usually exceed those of AD, the highest physiological plasma concentrations of NA reported for dogfish (450 nmol l^{-1} in acute hypoxia, Table 2) are insufficient to produce more than 5 % of the maximum response to NA.

The results of the present study suggest that the adrenergic control of the dogfish heart is mediated by circulating AD. The upper range of AD concentrations in dogfish plasma are sufficient to elicit up to 60–90 % of the maximum response of the heart to catecholamines, whereas even the highest NA concentrations reported for dogfish plasma produce negligible stimulation of heart rate or contractility. Therefore, although NA typically predominates in dogfish plasma (Table 2) and tissues (Ostlund, 1954; von Euler & Fange, 1961; Saetersdal *et al.* 1975; Abrahamsson, 1979), only AD appears to be sufficiently potent in stimulating the heart to do so at physiological concentrations.

Table 1. *$-\log EC_{50}$ values for the stimulation of rate and force of contraction of dogfish heart by catecholamines*

Agonist	Effect on myocardium		
	Sinus frequency	Atrial tension	Ventricular tension
Isoprenaline	7·7 ± 0·1 [20 nmol l^{-1}]	7·3 ± 0·2 [48 nmol l^{-1}]	7·5 ± 0·1 [30 nmol l^{-1}]
	NS	NS	*
Adrenaline	7·4 ± 0·2 [40 nmol l^{-1}]	7·0 ± 0·2 [107 nmol l^{-1}]	6·8 ± 0·3 [158 nmol l^{-1}]
	*	*	*
Noradrenaline	5·1 ± 0·1 [8 μmol l^{-1}]	4·9 ± 0·2 [14 μmol l^{-1}]	4·9 ± 0·2 [13 μmol l^{-1}]

$-\log EC_{50}$ values are expressed as their mean ± the standard error of the mean. Equivalent EC_{50} values are given in parentheses.

Number of observations used in calculation are given in Fig. 1.

Significant differences ($P < 0·05$) between adjacent values, as determined by analysis of variance and Duncan's multiple range test, are indicated by an asterisk (NS; not significant). There was no significant difference between $-\log EC_{50}$ values for drug effects on sinus frequency, atrial tension or ventricular tension.

Table 2. *Plasma adrenaline (AD) and noradrenaline (NA) concentrations in dogfish plasma under various conditions*

Species	Condition	AD	NA	Reference
Scyliorhinus canicula	At rest	6	14	Butler *et al.* 1986
	Spontaneous swimming	19	32	
	Burst swimming	96	97	
S. canicula	At rest	17	21	Butler *et al.* 1979
	Chronic hypoxia (72 h)	42	63	
S. canicula	At rest	26	32	Butler *et al.* 1978
	Acute hypoxia	284	447	
Squalus acanthias	At rest	6	9	Opdyke *et al.* 1982
	Tail grabbing	25	53	
S. acanthias	At rest	21	37	Opdyke *et al.* 1981
	After angiotensin II	96	250	
S. acanthias	1 min in air	55	30	Abrahamsson, 1979
	10 min in air	160	320	
	Killed by head trauma	1850	4080	

All values are in nmol l^{-1}.

The present study may also help clarify the manner in which the dogfish heart is influenced by catecholamines. The most pronounced effects of catecholamines in the present study were on the contractility of atrial and ventricular myocardium (300 and 200% maximum, respectively), with only small increases (20–40% maximum) being elicited in the frequency of spontaneous pacemaker activity. Therefore the adrenergic control of the dogfish heart *in vivo* should consist almost entirely of adjustments of the filling and contractility of the ventricle by changing plasma adrenaline concentrations. This suggestion is consistent with demonstrations that propranolol significantly reduces stroke volume and cardiac output in resting dogfish (Short *et al.* 1977), and that reflex adjustments of heart rate are mediated predominantly by vagal cholinergic mechanisms (Lyon, 1926; Short *et al.* 1977; Taylor *et al.* 1977).

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