# EVIDENCE FOR THE PRESENCE OF BOTH $A_1$ AND $A_2$ ADENOSINE RECEPTORS IN THE VENTRAL AORTA OF THE DOGFISH SHARK (SQUALUS ACANTHIAS)

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Our initial study of the effect of 2-chloroadenosine (A-CADO) on the vasoactivity of isolated rings from the ventral aorta of the dogfish demonstrated a slight, but significant (6%) vasoconstriction at concentrations below $10^{-5}$ M, but a more substantial vasodilation (20%) at 2-CADO concentrations above $10^{-5}$ M (Evans & Weingarten, Bull. MDIBL 28, 4-5, 1989), suggesting that both $A_1$ and $A_2$ receptors may be present, as has been described for a variety of tissues (e.g., Stiles, TIPS Dec. 1986, 486-490; Williams, Neurochem. Int. 14, 249-264, 1989) including the shark rectal gland (Forrest et al., Bull. MDIBL 20, 152-155, 1980; Kelley et al., Bull. MDIBL 23, 86-88 1983). An earlier study (Colin et al., J. Comp. Physiol. 130, 325-330, 1979) had demonstrated a vasodilatory action of $10^{-6}$ M adenosine on the trout gill vasculature, but, to date, no detailed study of the presence of $A_1$ vs. $A_2$ receptors in fish vascular smooth muscle has been published. It is now generally accepted that one can distinguish between two adenosine receptors either by the effect of a given agonist on the cytoplasmic cAMP concentrations ($A_1$ inhibitory, $A_2$ stimulatory) or by a structure-activity relationship (SAR) of CPA > R-PIA > 2 CADO > NECA > S-PIA ($A_1$) vs. NECA > 2 CADO > R-PIA > CPA > S-PIA ($A_2$) (e.g., Williams, op. cit., 1989). We therefore decided to examine the sensitivity of the dogfish ventral aortic vascular smooth muscle (VSM) to these agonists.

The preparation and mounting of dogfish aortic VSM rings for tension measurements has already been described (Evans & Weingarten, Bull. MDIBL 27, 84-85, 1987-88). 10 mM stock solutions of the agonists were prepared in the following way, before dilution in the experimental baths to attain the desired concentration: 2 CADO (distilled $H_2O$), CPA ($N^6$-Cyclopentyl-adenosine; dissolved first in 200 µl DMSO, then made up to 10 ml with distilled water), NECA (5'-N-Ethylcarboxamidoadenosine; distilled water), R-PIA ($N^6$-(2-Phenylisopropyl) adenosine, R (-) isomer; dissolved in 200 µl DMSO, then made up to 6.5 ml with distilled water), S-PIA (S (+) isomer (300 µl DMSO, 6.5 ml distilled water). Under our experimental conditions, the maximal DMSO concentration which the rings might experience would be 0.03%; pilot studies with 3% DMSO demonstrated no vasoactivity, so we are convinced that the carrier did not have any effect on our system. Initial tension of the rings was 504 ± 3.8 mg (N =38).

TABLE 1 The effect of various adenosine agonists on contraction of aortic vascular smooth muscle from Squalus acanthias

| Agonist | $10^{-8}$ M | $10^{-7}$ M | $10^{-6}$ M | $10^{-5}$ M | $10^{-4}$ M |
|---|---|---|---|---|---|
| 2 CADO (9) | 0* | 1.0±1.3 | 11±7.7** | -1.0±6.6 | -55±16** |
| CPA (6) | 1.7±3.3 | 5.8±4.6** | 15±5.3** | 17±6.0** | 17±6.0** |
| NECA (10) | 4.5±4.8 | 8.0±5.8 | 20±6.6 | 37±18 | 46±23 |
| R-PIA (10) | 4.5±2.0 | 6.0±2.5 | 13±3.8** | 20±6.3** | 3.5±3.4*** |
| S-PIA (3) | 0±2.9 | 1.7±4.4 | 4.3±4.3 | 4.3±4.3 | -23±12 |

* Mean ± S.E. of change in tension (mg) from unstimulated state
** $p < 0.05$ when compared to tension at $10^{-8}$ M
*** $p < 0.05$ when compared to tension at $10^{-5}$ M
(n) = number of rings tested

It is clear that these adenosine agonists can produce significant, albeit rather small, changes in vascular tension in this preparation. In the case of the putative $A_1$

receptor, they do so in an SAR similar to that described for other adenosine-sensitive tissues: CPA > R-PIA > 2 CADO > NECA > S-PIA, the classic SAR for an $A_1$-type receptor (see above). However, the degree of vasoconstriction is quite small, and even the best $A_1$ agonist, CPA, appears to have an $EC_{50}$ well above $10^{-7}$ M, far above the usual nanomolar $EC_{50}$. Only 2 CADO produced significant vasodilation, and then only at concentrations above $10^{-5}$ M (vasodilation at $10^{-3}$ M was -81 ± 12 mg (4)). Interestingly, NECA is apparently rather inactive in this tissue, not producing any measurable vasodilation, even at $10^{-4}$ M. R-PIA ($10^{-4}$ M) did produce some vasodilation, when compared to $10^{-5}$ M, but far less than 2 CADO. S-PIA did not produce a significant vasodilation at even high concentrations because of the small sample size, but all three rings had reduced tension at $10^{-4}$ M. CPA did not produce any vasodilation, even at the highest concentration used. We would tentatively conclude that the putative $A_2$ receptor has the SAR of 2 CADO > R-PIA > S-PIA > CPA = NECA, distinctly different from that described for the classic $A_2$ receptor (see above).

In summary, these results support the conclusion that the shark ventral aorta possesses both $A_1$ and $A_2$ adenosine receptors. However, its sensitivity to the classic agonists is distinctly lower than mammalian tissues, and standard SARs do not necessarily hold. The physiological significance of these purinergic receptors in the control of shark branchial circulation is unknown, but could play a role in gas exchange, osmoregulation, acid-base regulation, and ammonia excretion, since these are all important functions of the elasmobranch gill epithelium. Supported by NIH EHS-P30-ESO3828 to the Center for Membrane Toxicity Studies and NSF DCB-8801572 to DHE.