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COMMENTARY

Alpha-2 adrenoceptor subtypes: are more better?

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The discovery of an additional duplicated alpha-2 adrenoceptor subtype in the zebrafish raises a pesky nomenclature issue, as well as questions about the functions of the alpha-2 adrenoceptors in the zebrafish and how many alpha-2 receptors does an organism really need.
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Adrenergic receptors were originally divided into two major types, alpha and beta. Based on both pharmacological and molecular evidence, they are now divided into three major types – alpha-1, alpha-2 and beta – each of which is further subdivided into three (or more) subtypes (Bylund, 1988). Evidence for alpha-2 adrenoceptor subtypes arose first from radioligand binding and functional studies in mammalian systems, and the existence of the three subtypes defined by these studies was confirmed by molecular cloning in several species (for references see Bylund *et al*., 1994; Alexander *et al*., 2004). The three alpha-2 adrenergic receptor subtypes have now been well characterized in mammalian species and are identified as the alpha-2A, alpha-2B and alpha-2C adrenoceptors (Bylund *et al*., 1994). In mammalian species, the alpha-2A subtype is the main subtype in most brain regions. The alpha-2C subtype is found in particularly high concentrations in the caudate, whereas the alpha-2B subtype has a more limited distribution.

The pharmacological characteristics of the three subtypes are remarkably consistent across different species with the notable exception of the alpha-2A. This subtype has one pharmacological profile in man, dog, rabbit, pig and chicken, but a different profile in rat, mouse and cow. As a result of these significant pharmacological differences, and the widespread use of the rat and mouse as model systems, many investigators find it useful to retain the alpha-2D nomenclature originally assigned to these alpha-2A adrenoceptors. An important, but not the only, pharmacological difference between the alpha-2A and the alpha-2D is the lower affinity of yohimbine and rauwolscine for the alpha-2D (Link *et al*., 1992; O'Rourke *et al*., 1994).

The paper by Ruuskanen *et al*. (in press) in this issue expands our understanding of the pharmacology of the alpha-2 adrenoceptors by presenting a detailed description of the five alpha-2 adrenoceptors found in the zebrafish. Three of the subtypes are similar to those found in mammals (orthologs – same gene in different species), whereas the other two are not found in mammals, but are paralogs (duplicated genes in the same species). Interestingly, the pufferfish may contain as many as eight alpha-2 subtypes (the five found in the zebrafish plus paralogs of the alpha-2A, alpha-2B and alpha-2C subtypes; Ruuskanen *et al*., 2004). The two new zebrafish subtypes have been named alpha-2Da and alpha-2Db, which, although perhaps correct, will unavoidably cause confusion with the mammalian pharmacologically-defined alpha-2D. The confusion will be somewhat reduced because the zebrafish alpha-2A receptors appear to have pharmacological characteristics similar to the human, rabbit and pig, rather than the alpha-2D pharmacology of the rat, mouse and cow (see Table 1). It has been suggested that the residue equivalent to the cystine at position 201 in the human is an important determinant of alpha-2A vs alpha-2D pharmacology (Link *et al*., 1992), and the zebrafish alpha-2A receptor does have a cystine at this position, as does the pig. However, the chicken, which also has alpha-2A pharmacology, has a serine at this position similar to the rat, cow and mouse, which have alpha-2D pharmacology. Interestingly, the nucleotide sequence for the chicken and cow at this position are similar (agc) but different from the rat and mouse (tcc). Both are just one base different from the tgc codon for cystine. In any case, because the chicken has a serine at the human 201 position and also clearly has alpha-2A pharmacology, this residue cannot be critical in determine alpha-2A vs alpha-2D pharmacological characteristics.

Ruuskanen *et al*. (in press) chose a reasonable set of 20 ligands for a pharmacological comparison of the subtypes in the zebrafish. They point out that there are only small differences in affinity among the same subtype in different species, whereas there are larger differences among the subtypes. Perhaps this is to be expected because many of the ligands chosen (oxymetazoline, rauwolscine, yohimbine, chlorpromazine, ARC239, prazosin, spiroxtrine, WB-4104) have been used frequently used in alpha-2 studies precisely because their affinities differ among subtypes. There may well be other ligands that will eventually be found that will differentiate the zebrafish receptors from their mammalian orthologs. However, from the pharmacological perspective of using other species to model the human, it is convenient that drugs generally appear to have similar affinities across orthologs. Zebrafish are rapidly becoming an important tool in drug discovery, because they combine the relevance of a vertebrate with the scalability of an invertebrate (Goldsmith, 2004).

The presence of an additional duplicated subtype, the alpha-2Da and alpha-2Db, makes one wonder how many total adrenoceptors the zebrafish has. So far, the only other adrenoceptor that has been documented in the zebrafish is

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Table 1 Pharmacological comparison of alpha-2A receptors in various species

	Alpha-2A pharmacology					*Alpha-2D pharmacology*		
	Human[a]	*Zebrafish*[a]	*Rabbit*[b]	*Pig*[c]	*Chicken*[d]	*Rat*[b]	*Cow*[c]	*Mouse*[b]
ARC239	2100	1800	100,000	365	133	749	352	484
Rauwolscine	1.9	1	11.3	0.37	0.25	19	9.5	64
Ratio	1105	1800	8850	986	605	39	37	8

The ratios of the radioligand binding K_i values for ARC239 and rauwolscine illustrate the marked pharmacological differences between the alpha-2A (human, rabbit, pig, chicken) and the alpha-2D ortholog subtypes (rat, cow, mouse). The zebrafish has alpha-2A pharmacology.
[a]Ruuskanen *et al*. (in press).
[b]Naselsky *et al*. (2001).
[c]O'Rourke *et al*. (1994).
[d]Bylund *et al*. (1988).

the alpha-1A. However, if we assume that there are at least three alpha-1 and three beta adrenoceptors, which would bring the total number of adrenoceptors to 11 in the zebrafish (and perhaps to as many as 14 in the puffferfish), one cannot help asking how many adrenoceptors in general and how many alpha-2 adrenoceptors in particular does an organism need and what physiological functions do they subserve? Before these questions can be approached, some information is needed regarding the presence and function of epinephrine and norepinephrine in the zebrafish. Similar to mammals, the locus coeruleus in the zebrafish provides most, if not all, of the noradrenergic inputs to brain regions, and projection patterns and terminals of individual cell groups have been studied (Ma, 1997). In fishes, the catecholamines have been linked to a variety of behaviors, including aggression and social dominance, courtship and reproductive behavior (Ma, 1997). However, specific functions for alpha-2 adrenoceptors in the zebrafish are unknown.

Just as in mammals, all zebrafish alpha-2 receptors have similar affinities for epinephrine and norepinephrine, indicating that this is probably not a main reason for subtypes. In mammals, it has been suggested that the differential localization and regulation of the alpha-2 subtypes might be important for the three subtypes, and it will be of interest to see if this extends to the zebrafish.

Ruuskanen *et al*. (in press) show that the zebrafish alpha-2 adrenoceptors can couple to mammalian G proteins that are sensitive to pertussis toxin, and thus these receptors are 'functional' – at least in a mammalian system. Owing to our recent understanding of agonist trafficking of alpha-2-mediated responses (Pauwels *et al*., 2001), and that the efficacy of agonist is very dependent on the system in which it is assessed (Kenakin, 2002), these GTPγS binding studies in CHO cells, although useful, shed little light on the actual function of these receptors in the zebrafish.

References

ALEXANDER, S.P.H., MATHIE, A. & PETERS, J.A. (2004). Guide to receptors and channels; adrenoceptors, α_2. *Br. J. Pharmacol.,* **141** (Suppl. 1), S9.

BYLUND, D.B. (1988). Subtypes of α_2-adrenoceptors: pharmacological and molecular biological evidence converge. *Trends Pharmacol. Sci.,* **9,** 356–361.

BYLUND, D.B., EIKENBERG, D.C., HIEBLE, J.P., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P., MOLINOFF, P.B., RUFFOLO, R.R. & TRENDELENBURG, A.U. (1994). IV. International Union of Pharmacology Nomenclature of Adrenoceptors. *Pharmacol. Rev.,* **46,** 121–136.

BYLUND, D.B., RUDEEN, P.K., PETTERBORG, L.J. & RAY-PRENGER, C. (1988). Identification of α_2-adrenergic receptors in chicken pineal gland using [^{3}H]rauwolscine. *J. Neurochem.,* **51,** 81–86.

GOLDSMITH, P. (2004). Zebrafish as a pharmacological tool: the how, why and when. *Curr. Opin. Pharmacol.,* **4,** 504–512.

KENAKIN, T. (2002). Drug efficacy at G protein-coupled receptors. *Annu. Rev. Pharmacol. Toxicol.,* **42,** 349–379.

LINK, R.E., DAUNT, D.A., BARSH, G.S., CHRUSCINSKI, A.J. & KOBILKA, B.K. (1992). Cloning of two mouse genes encoding alpha-2 adrenergic receptor subtypes and identification of a single amino acid in the mouse alpha-2C10 homolog responsible for an interspecies variation in antagonist binding. *Mol. Pharmacol.,* **42,** 16–27.

MA, P.M. (1997). Catecholaminergic systems in the zebrafish. III. Organization and projection pattern of medullary dopaminergic and noradrenergic neurons. *J. Comp. Neurol.,* **381,** 411–427.

NASELSKY, D.P., ASHTON, D., RUFFOLO JR, R.R. & HIEBLE, J.P. (2001). Rabbit α_2-adrenoceptors: both platelets and adipocytes have α_{2A}-pharmacology. *J. Pharmacol. Exp. Ther.,* **298,** 219–225.

O'ROURKE, M.F., IVERSEN, L.J., LOMASNEY, J.W. & BYLUND, D.B. (1994). Species orthologues of the alpha-2A adrenergic receptor: the pharmacological properties of the bovine and rat receptors differ from the human and porcine receptors. *J. Pharmacol. Exp. Ther.,* **271,** 735–740.

PAUWELS, P.J., TARDIF, S., COLPAERT, F.C. & WURCH, T. (2001). Modulation of ligand responses by coupling of α_{2A}-adrenoceptors to diverse G_α-proteins. *Biochem. Pharmacol.,* **61,** 1079–1092.

RUUSKANEN, J.O., LAURILA, J., XHAARD, H., RANTANEN, V.-V., VUORILUOTO, K., WURSTER, S., MARJAMÄKI, A., VAINIO, M., JOHNSON, M.S. & SCHEININ, M. Conserved structural, pharmacological and functional properties among the three human and five zebrafish a_2-adrenoceptors. *Br. J. Pharmacol.,* (in press).

RUUSKANEN, J.O., XHAARD, H., MARJAMAKI, A., SALANECK, E., SALMINEN, T., YAN, Y.L., POSTLETHWAIT, J.H., JOHNSON, M.S., LARHAMMAR, D. & SCHEININ, M. (2004). Identification of duplicated fourth α_{2A}-adrenoceptor subtype by cloning and mapping of five receptor genes in zebrafish. *Mol. Biol. Evol.,* **21,** 14–28.

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