
The Quantitative Difference in Adrenergic Alpha-1, Alpha-2 and Beta Receptors between the Prostatic and Epididymal Ends of Dog Vas Deferens

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The Second Department of Biochemistry and *the Second Department of Internal Medicine, Akita University School of Medicine, Akita 010 and +Department of Urology, Tokyo Medical and Dental University School of Medicine, Tokyo 113

KONDO, S., KAGAYA, M., TASHIMA, Y. and MORITA, T. The Quantitative Difference in Adrenergic Alpha-1, Alpha-2 and Beta Receptors between the Prostatic and Epididymal Ends of Dog Vas Deferens. Tohoku J. Exp. Med., 1992, 167 (2), 167-169 — In order to study the regional differences in the distribution of adrenergic receptors of the vas deferens, we measured the amounts of adrenergic α-1, α-2 and β receptors in prostatic, intermediate, and epididymal portions of the dog vas deferens using radioligand binding techniques. Saturation experiments with 3H-prazosin, 3H-yohimbine, and 3H-dihydroxyalprenolol demonstrated that there were significant amounts of adrenergic α-1, α-2, and β receptors, respectively, in these three portions. α-1 receptors existed most densely in the whole vas deferens. The prostatic portion was found to have the largest amounts of α-1 and α-2 receptors among three portions studied. β-Receptors were distributed most densely in the intermediate portion. α-1, α-2, and β adrenergic receptors show different distribution patterns throughout the whole length of vas deferens.

α-1 receptor; α-2 receptor; β-receptor; dog vas deferens

MacDonald and McGrath (1980) reported that there were regional differences in the response to various autonomic drugs between the prostatic and epididymal ends of the vas deferens; their results suggest that there are differences in the distribution of autonomic receptors. However, there has been no investigation on the quantitative analysis of the differences in the amount of autonomic receptors in the vas deferens. Therefore, we measured adrenergic α-1, α-2 and β receptors in the three portions (prostatic, intermediate and epididymal portions) of the dog vas deferens using radioligand binding techniques.

Adult mongrel dogs (body weight 20-30 kg) were anesthetized with pentobarbital sodium (Nembutal) and bled. Through a midline abdominal incision the whole length of bilateral vasa deferentia were removed. The specimens were transferred into cold saline, trimmed from adjacent connective tissues and were cut evenly into three portions. They were stored at −80°C until assay. The specimens from one dog were used for three types of assays using 3H-prazosin (3H-PZ), 3H-yohimbine (3H-YOH) and 3H-dihydroxyalprenolol

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(3H-DHA) as ligands for α-1, α-2 and β-receptors. Five animals were used in the experiments.

The frozen tissue was thawed and homogenized in 50 volumes of ice-cold 50 mM Tris-HCl buffer (pH 8 at 25°C) for two 20-sec periods with a 10-sec interval, using a Polytron PT 35 at speed 6 with probe generator PT 10/35 (Brinkman Instruments Inc., Westbury, NY, USA). The homogenate was centrifuged at 49,000 × g for 15 min at 4°C in a Sorvall RC 5-B (DuPont Instruments, Boston, MA, USA). The pellet was resuspended and homogenized with 50 volumes of 50 mM Tris-HCl buffer (pH 8.0), filtered through a 100-μm pore nylon mesh and centrifuged as before. The resultant pellet was resuspended in appropriate volumes of incubation buffer and tissue concentration was adjusted appropriately for each binding assay. The tissue dilutions, total incubation volumes and incubation buffers in the final membrane mixture for various 3H-ligands were as follows; for 3H-PZ, 80 volumes, 1 ml and, 25 mM glycylglycine, pH 7.4; for 3H-YOH, 40 volumes, 0.5 ml, and 25 mM glycylglycine, pH 7.4; for 3H-DHA, 80 volumes, 1 ml, and Tris-HCl, pH 8.0.

Saturation experiments were performed in order to calculate the maximum number of binding sites (Bmax) and the equilibrium dissociation constant (Kd) for each receptor type. Aliquots of membrane preparations were incubated for 30 min (for 3H-PZ and 3H-YOH) and 45 min (for 3H-DHA) at 23°C with increasing concentrations of labeled ligand. At the end of the incubation period, the mixture were filtered through Whatman GF/B filter papers using a manifold cell harvester (Brandel Co., Gaithersberg, MD, USA) and each filter paper was washed with 8 to 10 ml of ice-cold 50 mM Tris-HCl buffer (pH 8.0, 25°C). The filter papers were kept soaked in 0.05% polyethylenimine solution for 60 min before filtration in order to reduce the non-specific binding to the filter paper. The filter papers were transferred into scintillation vials containing 10 ml of toluene-based scintillation fluid. The vials were shaken for 1 hr and the radioactivity was counted in a liquid scintillation counter at an efficiency of 50-55%. The specific binding was defined as the difference between the bindings in the presence and absence of an excess amount of an appropriate unlabeled ligand. (-)-Norepinephrine (10-4 M) was used to determine the non-specific binding for 3H-PZ and 3H-YOH. In our preliminary experiments norepinephrine was found to give less non-specific binding than phentolamine. (±)-Propranolol (10-6 M) was used to determine the non-specific binding for 3H-DHA.

Saturation data were calculated according to Rosenthal analysis (1967) using linear

<table>
<thead>
<tr>
<th>Portions of dog vas deferens</th>
<th>3H-PZ</th>
<th>3H-YOH</th>
<th>3H-DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostatic</td>
<td>81.9 ± 23.6*</td>
<td>31.8 ± 7.5</td>
<td>33.4 ± 8.9*</td>
</tr>
<tr>
<td>Intermediate</td>
<td>12.3 ± 0.54</td>
<td>17.2 ± 2.2</td>
<td>22.4 ± 2.3</td>
</tr>
<tr>
<td>Epididymal</td>
<td>0.74 ± 0.48</td>
<td>12.4 ± 1.4</td>
<td>9.8 ± 3.2</td>
</tr>
</tbody>
</table>

*Values are means ± S.D. of five determinations. Saturation curves are obtained from incubation of membranes with increasing concentrations of 3H-ligands. The same data are plotted for linear regression according to Rosenthal (1967). Bmax is obtained from the intercept with the abscissa and Kd is calculated from the negative reciprocal of the slope of the line.

*p < 0.05 compared to different portions of vas deferens.
regression of bound/free vs. bound in order to calculate Bmax and Kn values. For statistical analysis a non-paired Student's t-test was used (p<0.05 as significant). Protein concentration in the particulate fraction was measured by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Saturation experiments revealed the presence of a single class of saturable high affinity binding sites for \(^3\)H-PZ, \(^3\)H-YOH, and \(^3\)H-DHA in all the specimens examined. Table 1 shows the results of saturation experiments of bindings of the three \(^3\)H-ligands to the three portions of the dog vas deferens. The Kd values for the three \(^3\)H-ligands were not significantly different among three portions of the vas deferens. The Bmax values for \(^3\)H-PZ and \(^3\)H-YOH were highest in the prostatic portion, and that for \(^3\)H-DHA was highest in the intermediate portion. In the whole length of vas deferens, alpha-1 receptors existed most densely. The density of alpha-1 and alpha-2 receptors was higher in the prostatic portion than in the other two portions. The highest density of beta-receptors was observed in the intermediate portion. These differences were statistically significant.

Hay and Wadsworth (1983) showed that alpha-adrenoceptor agonists produce an initial tonic response followed by rhythmic contractions in the vas deferens. This response was explained to be mediated by both alpha-1 and alpha-2 adrenoceptors (Lafi and Leake 1986). MacDonald and McGrath (1980) reported the existence of inhibitory beta receptors in the vas deferens. On the basis of the pharmacological data they explained that the subtype of these $\beta$-receptors was $\beta$-2. In the present study beta receptors were labelled with \(^3\)H-DHA, which cannot distinguish between beta receptor subtypes. For the measurement of $\beta$ receptor subtype $^{125}\text{I}$-ligands are available. However, studies with those ligands were not able to be included in the present study because of limited amount of specimens. Our data show that there exist significant amounts of $\alpha$-1, $\alpha$-2 and $\beta$ receptors in dog vas deferens. Both adrenergic alpha and beta receptors are thought to regulate the contractions of the vas deferens. It is not clear how the different pattern of the distribution of adrenergic receptors correlates with the vasal motility. It is known that the vasal contents are transported not only distally but also proximally (Ewing and Chang 1986). The existence of the density gradient of adrenergic receptors may play some roles in the mode of vasal contractions. Adrenergic regulation of the function of the vas deferens needs further study.

References