SPECIFIC $^3$H-HALOPERIDOL BINDING TO DOPAMINE RECEPTORS IN THE ANTERIOR BYSSUS RETRACTOR MUSCLE OF MYTILUS EDULIS

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Abstract—The anterior byssus retractor muscle (ABRM) of Mytilus edulis has specific dopamine receptors. We carried out a radioligand binding assay for dopamine receptors in ABRM using ($^3$H)-haloperidol as the radioligand. High affinity binding of ($^3$H)-haloperidol has been shown. Scatchard analysis showed a single component of binding with an apparent equilibrium constant ($K_d$) of 1.6 nM and a maximal number of binding sites (Bmax) of 219 fmoles/mg protein. Some dopamine antagonists displaced 3 nM ($^3$H)-haloperidol binding, and the IC50 and Ki-value of these drugs were calculated. Considering these results, this muscle is thought to be suitable for a study of the dopamine receptors.

Dopamine relaxes catch contraction in the anterior byssus retractor muscle (ABRM) of Mytilus edulis and is present in the ganglia of Mytilus (1, 2). However, little is known about the dopamine receptors in this muscle. We have already investigated some properties of the dopamine receptors in ABRM (3), and these results indicated that relaxation of catch contraction in ABRM by dopamine is mediated through dopamine receptors, but not through adrenoceptors. Therefore, if dopamine receptors in ABRM can be shown to be similar to those in the mammalian brain, then such an isolated preparation with a directly observable physiological response could provide an useful model for the screening of dopaminergic agonists and antagonists. Such a model would be useful because there are no well characterized peripheral dopamine receptors in mammalian preparations. In this study, we have characterized the dopamine receptors in ABRM using a radioligand binding assay.

MATERIALS AND METHODS

Sea mussels, Mytilus edulis L., collected from the east side of Tokyo bay were used. Mytilus edulis were stored in aerated artificial sea water at a temperature of about 10°C and used within one week after collection. According to de Rome et al. (4), the anterior byssus retractor muscle (ABRM) of Mytilus edulis was removed, weighed and minced with a pair of scissors. Using a polytron (Kinematic GmbH), the minced preparation was homogenized in 40 volumes of ice cold 50 mM Tris/HCl, pH 7.4, that contained 0.1% ascorbic acid, 10 μM pargyline and 0.1 mM EGTA. The homogenate was centrifuged at 40,000×g for 10 min. The pellet was resuspended in the same volume of buffer and repelleted under the same conditions. After the pellet was again resuspended in 40 volumes of the same buffer and in-
cubated at 25°C for 5 min to allow monoamine oxidase inhibition by pargyline (5), aliquots were used in the binding assay. Assay tubes contained 3 nM 3H-haloperidol (12.5 Ci/mmol, New England Nuclear), homogenate, the drug being tested, and the appropriate buffer to a final volume of 1.2 ml. Tubes containing excess unlabelled haloperidol (1 μM) were used to determine "non-specific" binding. Binding was initiated by the addition of the homogenate, and incubation was carried out at 25°C for 30 min with shaking. The samples were then filtered through Whatman GF/C filters under vacuum, washed twice with 5 ml of ice-cold Tris/HCl buffer (pH 7.4) and counted in a liquid scintillation counter (Aloka LSC-900). Specific binding was obtained by subtracting the nonspecific binding in the presence of 1 μM haloperidol from the total bound radioactivity.

The protein concentration in the incubation medium was about 0.5 mg protein/ml which was determined by the method of Lowry et al. (6) using bovine serum albumin as the standard.

Drugs used: EGTA (Wako Pure Chemical Industry), Tris (Sigma), pargyline HCl (Sigma), ascorbic acid (Wako Pure Chemical Industry), haloperidol (Dainihon Seiyaku), Domperidone (Kyowa Hakko), chlorpromazine (Yoshitomi Seiyaku) and trimetoquinol (Tanabe Seiyaku).

RESULTS

The binding of 3H-haloperidol to ABRM homogenates shown in this study. The incubation medium was chosen according to de Rome et al. (4) who reported that the presence of salts in heart membrane binding assays of bivalve mollusc significantly reduced specific binding and according to Bürki (7) who found that addition of EGTA to the reaction mixture stabilized 3H-haloperidol binding. The incubation was carried out for 30 min since the binding was stabilized from 30 to 60 min. Figure 1 shows a saturation curve of 3H-haloperidol binding in the concentration range of 0.5 to 10 nM. Scatchard analysis (Fig. 2) shows a single component of binding with an apparent equilibrium dissociation constant (Kd) of 1.6 nM and a maximal number of binding sites (Bmax) of 219 fmoles/mg protein.

From the curves for the inhibition of specific 3H-haloperidol binding by some dopamine antagonists (Fig. 3), inhibition constants were calculated (Table 1). Trimetoquinol, a beta-adrenoceptor stimulant with a dopamine moiety, was reported not to have dopamine-like activity (namely, relaxation of catch contraction). However, it was found to have antidopamine activity in this muscle (8). A high concentration of trimetoquinol inhibited the specific 3H-haloperidol binding. The Ki values for dopamine antagonists obtained from the inhibition of 3H-haloperidol binding were 1.7 nM (haloperidol), 40.5 nM (do-
DISCUSSION

In the ABRM of Mytilus edulis, dopamine relaxes the catch contraction (1, 2). We reported previously that the relaxation of catch by dopamine was mediated through the dopamine receptors, but not through the adrenoceptors (3). However, little is known about the dopamine receptors in ABRM. Therefore, we studied the dopamine receptors.

There was a specific 3H-haloperidol binding in ABRM homogenates. The binding was saturable and of a high affinity. In the preliminary experiment, we tried a 3H-dopamine binding assay. The binding of 3H-dopamine on filters in the absence of protein was nearly the same value with increasing concentration of cold ligands when the concentration of 3H-dopamine was low (10^{-9} M); but when the concentration of 3H-dopamine was above 10^{-8} M, the binding on filters decreased with increasing concentrations of cold ligands. Moreover, binding of 3H-dopamine in the presence of protein

![Fig. 2. Scatchard plot of specific 3H-haloperidol binding in the homogenates of ABRM from Fig. 1. Each point represents the mean of two independent experiments. The line was obtained by the method of least squares. The slope of the plot, -1/Kd, was determined by linear regression analysis; and the number of binding sites, Bmax, computed from the intercept of the plot with the abscissa. Abscissa: concentration of 3H-haloperidol (bound), pmol/mg protein; ordinate: the ratio of bound- to free-3H-haloperidol found in the incubation medium. Concentrations of free 3H-haloperidol were evaluated by subtracting total bound 3H-haloperidol from 3H-haloperidol added in the incubation medium.](image)

![Fig. 3. Inhibition of specific 3H-haloperidol binding to ABRM homogenates by some dopamine antagonists. The concentration of 3H-haloperidol was 3 nM. Abscissa: concentration of drugs (log scale), ordinate: specific 3H-haloperidol bound (%). Each value represents the mean with S.E. of three different experiments.](image)
Table 1. Inhibition of specific $^3$H-haloperidol binding to ABRM homogenates by some dopamine antagonists. $K_i$ values were calculated from the IC50 values of the drugs according to the relationship $K_i = IC50/1 + c/K_D$, where $K_D = 1.6$ nM and $c$ is the concentration of $^3$H-haloperidol (3 nM). IC50 values were determined from the plots of Fig. 3.

| Drug            | IC50 (nM) | $K_i$ (nM) |
|-----------------|-----------|------------|
| Haloperidol     | 4.6       | 1.7        |
| Domperidone     | 112       | 40.5       |
| Chlorpromazine  | 166       | 60.0       |
| Trimethoquinol  | 2880      | 1040       |

decreased with increasing concentrations of cold ligands, but we could not determine the concentration of cold ligand at which the binding stabilized. The reason why the $^3$H-dopamine displacement curve was complicated is unclear in this study, and further studies must be done on the medium or the preparation of homogenate.

The ratio of specific $^3$H-haloperidol binding to total $^3$H-haloperidol binding was relatively low. One of the reasons for this might be that this homogenate contained a rather large number of nonspecific binding sites for $^3$H-haloperidol.

Some dopamine antagonists inhibited $^3$H-haloperidol binding (3 nM). We calculated the IC50 and $K_i$ values from the inhibition curves of these drugs. The obtained IC50 values of haloperidol and chlorpromazine were similar to those of the rat striatum particulate fraction (9), and the $K_D$ value was similar to that of calf caudate membrane (10). On the other hand, we calculated the $K_D$ value of dopamine using a photoaffinity labeling technique (11). The affinity of dopamine to ABRM was very low since the estimated $K_D$ value was 1.1 nM (12). This value coincided with the $K_i$ value for dopamine against the specific binding of $^3$H-domperidone to mouse striatal membranes (13). The activation of adenylate cyclase by dopamine could not be observed in this muscle (unpublished data), so the type of dopamine receptors in ABRM appears to somewhat resemble Seeman’s D-2 receptors (14). However, further studies of the dopamine receptors in ABRM are necessary in order to decide the type of the receptors.

REFERENCES

1) Hidaka, T., Yamaguchi, H., Twarog, B.M. and Muneoka, Y.: Neurotransmitter action on the membrane of Mytilus smooth muscle-II. Dopamine. Gen. Pharmacol. 8, 87-91 (1977)
2) Twarog, B.M., Muneoka, Y. and Ledgere, M.: Serotonin and dopamine as neurotransmitters in Mytilus: Block of serotonin receptors by an organic mercurial. J. Pharmacol. Exp. Ther. 201, 350-356 (1977)
3) Takayanagi, I., Murakami, H., Iwayama, Y., Yoshida and Miki, S.: Dopamine receptor in anterior byssus retractor muscle of Mytilus edulis. Japan. J. Pharmacol. 31, 249-252 (1981)
4) de Rome, P.J., Jamieson, D.D., Taylor, K.M. and Davies, L.P.: Ligand-binding and pharmacological studies on dopamine and octopamine receptors in the heart of the bivalve mollusc Tapes watlingi. Comp. Biochem. Physiol. 67C, 9-16 (1980)
5) Burt, D.R., Enna, S.J., Creese, I. and Snyder, S.H.: Dopamine receptor binding in the corpus striatum of mammalian brain. Proc. Natl. Acad. Sci. U.S.A. 72, 4655-4659 (1975)
6) Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275 (1951)
7) Bürki, H.R.: Correlation between $^3$H-haloperidol binding in the striatum and brain amine metabolism in the rat after treatment with neuroleptics. Life Sci. 23, 437-442 (1978)
8) Yoshida, Y., Takayanagi, I. and Murakami, I.: Dopamine and its antagonists on molluscan smooth muscle. J. Pharmacobiodyn. 4, 226–228 (1981)

9) Laduron, P.M. and Leysen, J.E.: Domperidone, a specific in vitro dopamine antagonist, devoid of in vivo central dopaminergic activity. Biochem. Pharmacol. 28, 2162–2165 (1979)

10) Burt, D.R., Creese, I. and Snyder, S.H.: Properties of [3H]haloperidol and [3H]dopamine binding associated with dopamine receptors in calf brain membranes. Mol. Pharmacol. 12, 800–812 (1976)

11) Takayanagi, I., Yoshioka, M., Takagi, K. and Tamura, Z.: Photoaffinity labeling of the β-adrenergic receptors and receptor reserve for isoprenaline. Eur. J. Pharmacol. 35, 121–125 (1976)

12) Iwayama, Y. and Takayanagi, I.: Photoaffinity labeling of dopamine receptor in molluscan smooth muscle. J. Pharm. Pharmacol. 34 (1982) (in press)

13) Baudy, M., Martres, M.P. and Schwartz, J.C.: 3H-domperidone: A selective ligand for dopamine receptors. Naunyn Schmiedebergs Arch. Pharmacol. 308, 231–237 (1979)

14) Seeman, P.: Brain dopamine receptor. Pharmacol. Rev. 32, 229–313 (1981)