EFFECTS OF SOME ERGOT ALKALOIDS ON DOPAMINE RECEPTORS OF MOLLUSCAN SMOOTH MUSCLE

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Some ergot alkaloids relaxed catch contraction of an isolated molluscan smooth muscle (anterior byssus retractor muscle of Mytilus edulis). Haloperidol, a competitive dopamine antagonist, shifted the dose response curves of ergot alkaloids, but methysergide did not. Bromocriptine, a potent dopaminergic ergot alkaloid, did not relax catch contraction. These results suggest that relaxation of catch contraction by some ergot alkaloids is mediated through dopamine receptors of this muscle, but dopamine receptors of this muscle seem to be somewhat different from those of mammalian brain.

Keywords — dopamine receptors; ergot alkaloids; bromocriptine; haloperidol; anterior byssus retractor muscle

Dopamine relaxes catch contraction in anterior byssus retractor muscle (ABRM) of Mytilus edulis. We have already investigated a possible presence and some properties of dopamine receptors in ABRM. These results indicate that relaxation of catch in ABRM by dopamine is mediated through dopamine receptors but not through adrenoceptors.1) Radioligand binding assay showed a high affinity binding of [3H]haloperidol to the homogenate of ABRM.2) The calculated KD value of dopamine using a photoaffinity labeling technique was very high.3)

Recently Kebabian and Calne4) reported that several ergot derivatives could distinguish different dopamine receptors. According to them, the dopaminergic ergots, such as bromocriptine, lisuride and lergotrile, are extremely potent agonists of the dopamine receptor of the mammamorphs, but such ergots can block the striatal dopamine receptor linked to adenylate cyclase and extremely high concentrations of them can cause accumulation of cyclic AMP in some tissues. In order to know the type(s) of dopamine receptors in ABRM, we examined the effects of some ergot alkaloids.

Sea mussels, Mytilus edulis L., collected from the east coast of Tokyo Bay were used. Muscle bundle (about 1 mm in diameter) dissected from ABRM was suspended in a 10 ml organ bath filled with artificial seawater (NaCl 456, KCl 11, CaCl2 2H2O 11, MgCl2 6H2O 48 and Tris/HCl 25 mM; pH 7.8—8.0) bubbled with air and kept at 24 to 25°C. Responses to drugs were recorded isotonically under a tension of 0.2 g. After the muscle was exposed to acetylcholine (10^{-4} M) for 2 min to induce the catch contraction and washed with artificial seawater for 5 min, test drugs were applied. Relaxation by a 10 min exposure to the drugs was estimated. In order to estimate the maximum relaxation, serotonin (10^{-6} M) was applied after the muscle had been exposed to the test drug for 10 min. These relaxations were expressed as a percent of the maximum relaxation by serotonin (10^{-6} M). After a 2 min exposure to acetylcholine (10^{-4} M) and washing with artificial sea water, one of an-
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agonists was applied for 5 min and the test drug was given for 10 min in the presence of the antagonist.¹

Ergometrine, dihydroergotamine, ergotamine and α-ergocryptine relaxed catch contraction of this muscle, while bromocriptine (up to $3 \times 10^{-5}$ M) was ineffective (Fig. 1). The concentration-response curves of ergometrine and dihydroergotamine were shifted by haloperidol ($3 \times 10^{-5}$ M), a competitive dopamine antagonist, but those of ergotamine and α-ergocryptine were not significantly shifted. Ergometrine and dihydroergotamine are soluble in water, but other three ergot alkaloids are less soluble in water than the former two ergots. The solubility in water might differentiate the effectiveness for ABRM of ergot alkaloids investigated. None of ergot alkaloids investigated inhibited the response of dopamine at the concentrations that did not relax the catch contraction in ABRM.

Ergot alkaloids is known to interfere at more than one type of specific receptors (e.g. α-adrenoceptors, serotonin receptors and dopamine receptors). In ABRM serotonin relaxes the catch contraction in the same manner as dopamine does. Therefore, in order to know whether or not relaxation of catch by some ergot alkaloids is mediated by serotonin receptors, we studied the effects of methysergide ($1.7 \times 10^{-8}$ M), a competitive serotonin antagonist on the actions of ergot alkaloids. The concentration-response

**Fig. 1. Responses to Dopamine and Some Ergot Alkaloids in the Absence and Presence of Haloperidol**

Abscissa: negative log of concentration (M), ordinate: percent of the maximum relaxation by serotonin ($10^{-6}$ M). Solid line: agonist alone; dotted line: agonist with haloperidol ($3 \times 10^{-5}$ M). ●: dopamine; ○: ergometrine; ▲: dihydroergotamine; △: ergotamine; ▽: α-ergocryptine; ○: bromocriptine. a) significant difference from the corresponding control value at $p<0.01$; b) $p<0.05$. ED₅₀ (M): dopamine ($1.5 \times 10^{-8}$); ergometrine ($2.3 \times 10^{-8}$); dihydroergotamine ($2.9 \times 10^{-8}$); ergotamine ($3.0 \times 10^{-7}$ M); α-ergocryptine ($2.2 \times 10^{-6}$ M).

The muscle spontaneously relaxed to $23.0 \pm 2.5\%$ (mean ± S.E.) after exposure to acetylcholine and washing with artificial sea water. So spontaneous relaxation is presented as a horizontal dotted line. Each value is presented as a mean with S.E. of 4 to 8 experiments.

**Fig. 2. Responses to Serotonin and Some Ergot Alkaloids in the Absence and Presence of Methysergide**

Abscissa: negative log of concentration (M), ordinate: percent of the maximum relaxation by serotonin ($10^{-6}$ M). Solid line: agonist alone; dotted line: agonist with methysergide ($1.7 \times 10^{-8}$ M). ▼: serotonin; ○: ergometrine; ▲: dihydroergotamine, ○: ergotamine; ▽: α-ergocryptine. a) significant difference from the corresponding control value at $p<0.01$. Spontaneous relaxation is presented as a horizontal dotted line. Each value is presented as a mean with S.E. of 4 to 8 experiments.
the right, but those of ergot alkaloids were not (Fig. 2). These results suggest that in ABRM some ergot alkaloids do not interact with serotonin receptors, but interact with dopamine receptors.

According to Kebabian and Calne, a test to show whether dopamine can cause accumulation of cyclic AMP or not is helpful in knowing the type(s) of dopamine receptors in a certain tissue. We estimated cyclic AMP levels in ABRM. Isolated muscles were equilibrated for 1 h in test tubes filled with artificial sea water at room temperature and bubbled with air. After 2 min treatment with drug, muscles were frozen in liquid nitrogen. Cyclic AMP was assayed according to Steiner et al. The 2 min treatment of this muscle with serotonin (10^{-5} M) strikingly increased cyclic AMP level (untreated: 4.7±0.85; treated: 91.4±6.4 pmol/mg protein; n=5), but the 2 min treatment with dopamine (10^{-5} M) did not change cyclic AMP level significantly (untreated: 5.7±0.66; treated: 4.5±0.60; n=5).

On dopamine receptors in ABRM, some ergot alkaloids act as a dopaminergic agonist and the existence of dopamine receptors linked adenylate cyclase is not recognized. But bromocriptine which is known as a potent agonist of D-2 receptors could not interact with dopamine receptors in ABRM, and sulpiride which is known as a typical antagonist of D-2 receptors could not antagonize the effect of dopamine. In ABRM, apomorphine relaxed catch contraction at rather high concentrations and it did not antagonize the effect of dopamine. However, since apomorphine could not be antagonized by haloperidol (unpublished data), relaxation caused by apomorphine seems to be not mediated by dopamine receptors, but to be a non-specific action. Dopamine receptors in ABRM have some similarities to mammalian dopamine receptors, but are somewhat different from those of mammalian tissue. Further studies are needed to know the nature of dopamine receptors in ABRM.

REFERENCES