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Dopamine-sensitive adenylate cyclase in retina—Subcellular distribution

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A variety of evidence exists which implicates dopamine (DA) as a neurotransmitter in several areas of the mammalian brain [1]. Physiological and pharmacological evidence supports the concept of a "dopamine receptor" in these areas [2, 3]. Recent studies have demonstrated the presence of a dopamine-sensitive adenylate cyclase in various dopaminergic areas of the brain, including the caudate nucleus, olfactory tubercle, substantia nigra, nucleus accumbens and median eminence [4–11]. A dopamine-sensitive adenylate cyclase which displays many of the properties of that described for the central nervous system has also been observed in the retina [12–14].

In this study we demonstrate a specific subcellular localization of dopamine-sensitive adenylate cyclase in P_2 retinal fractions which have previously been shown to be the subcellular site of high affinity uptake and Ca^{2+} dependent, K^+ stimulated release of [^{3}H]DA [15].

ATP, cyclic AMP and EGTA* were purchased from Sigma Co., St. Louis, MO, and 3-hydroxytyramine (dopamine) was from CalBiochem, La Jolla, CA; inorganic salts were all reagent grade. All phenothiazines and related compounds were obtained, in high purity, from their commercial distributor.

Recently this laboratory has developed methods for isolating two synaptosomal fractions from rabbit retina [15, 16]. Retinas are homogenized; cell debris and nuclei are sedimented (pre-P_1 fraction) and discarded. A subsequent centrifugation produces a P_1 fraction which contains many large synaptosomes morphologically similar to photoreceptor terminals based on the following criteria: (1) unusually large size (3–4 μm diameter), (2) presence of invaginations containing presumptive post-/pre-synaptic membrane processes from horizontal and bipolar cells, and (3) presence of characteristic synaptic ribbons with an associated halo of synaptic vesicles. An additional fraction, the P_2 fraction, can also be obtained which has the morphological appearance of a P_2 fraction from whole brain. This fraction contains many small, conventional synaptosomes of less than 1 μm diameter. Some of these have portions of the post-synaptic membrane attached. Based on morphological similarities, these small, brain-like synaptosomes are presumably derived from amacrine, bipolar and horizontal cells. Analytical electron microscopic techniques [17] were used to quantitatively evaluate the content of various retinal fractions. Cross contamination of the two types of synaptosomes between the two fractions was calculated to be 10 per cent [15].

The retinas of adult, male, New Zealand white rabbits maintained on a 12-hr light/dark cycle with free access to food and water were used for these studies. During the light-adapted phase, the rabbits were decapitated and the eyes enucleated. All subsequent manipulations were carried out under ambient room light. A corneal transection was made and the cornea, iris, lens and vitreous were removed. The retina was teased from the underlying sclera and remaining attachments were severed. The isolated retinas were suspended in tubes containing ice-cold 0.32 M sucrose solution. All further fractionation procedures were carried out at 4°. The retinas were hand homogenized using five complete strokes with a Teflon pestle. The homogenate was centrifuged at 150 *g* for 10 min to remove a pre-P_1 fraction consisting of nuclei and cell debris. Low speed centrifugation (800 *g* for 10 min) of the above supernatant fluid produced a P_1 fraction containing photoreceptor cell synaptosomes. A P_2 fraction was obtained by centrifuging the resultant supernatant fluid at 25,000 *g* for 12 min. The P_2 fraction contained many small, conventional synaptosomes derived from the innerplexiform layer. The P_1 and P_2 pellets were resuspended in 2 mM Tris–2 mM EGTA for the determination of adenylate cyclase activity.

The standard assay mixture (final volume 0.5 ml) for measurement of adenylate cyclase activity contained (in m-moles/liter): Tris (hydroxymethyl) aminomethane-maleate, 80.2, pH 7.4; ATP, 0.3; $MgSO_4$, 2.0; theophylline, 10; EGTA, 0.6; 0.05 ml of tissue homogenate; and test substances as indicated. The enzyme was preincubated with all components of the standard assay system, except ATP, for 20 min at 4°; the reaction was initiated by the addition of ATP and carried out for 10 min at 30°. The reaction was terminated by boiling, and cyclic AMP was measured, as described previously [4, 5]. The data on adenylate cyclase activity are expressed as pmoles of cyclic AMP formed/mg of protein/10 min and represent mean values and ranges for three separate experiments. The standard error for the data herein was less than 10 per cent.

Protein was determined by the method of Lowry *et al.* [18].

Adenylate cyclase activity in subcellular fractions of the rabbit retina was observed to be present in both fractions. As shown in Table 1, the highest specific activity of adenylate cyclase was found in the P_2 fraction, which is enriched in synaptosomes from amacrine cells and perhaps to a lesser extent from bipolar and horizontal cells. Adenylate cyclase activity was also observed in the P_1 fraction, which contains synaptosomes from photoreceptor cells; however, adenylate cyclase sensitivity to dopamine was not associated with this fraction. Dopamine agonists, apomorphine, *N*-methyldopamine (epinine) and 2 amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) mimicked the action of dopamine on adenylate cyclase activity in retinal homogenates and subcellular fraction P_2.

A high degree of pharmacological specificity was demonstrated for the receptor–cyclase complex, as shown in Table 2. Chlorpromazine, a dopamine antagonist, competitively inhibited the activity of the enzyme in the P_2 fraction with a calculated inhibition constant of 5×10^{-8} M. A study of the relative effects of the *cis-trans* isomeric forms of fluphenthixol (a thioxanthene), showed that the α-isomer of fluphenthixol was a more potent antagonist of adenylate cyclase activity in the retina than the β-iosmer. In addition, the (+)-isomer of butaclamol was more potent than the (−)-isomer in blocking the stimulation of adenylate cyclase by dopamine. The inhibition constant for (+)-butaclamol was calculated to be 4.5×10^{-8} M.

Dopamine has been shown previously to be localized by histofluorescence [19] within the amacrine cells of the

* EGTA (Ethylene glycol-bis-(β-aminoethylether)-*N*,*N*′-tetraacetic acid).

Table 1. Effect of dopamine agonists on adenylate cyclase activity in subcellular fractions of the rabbit retina*

	Concn (μM)	Adenylate cyclase activity (pmoles cAMP/mg protein/10 min)		
		Homogenate	P_1 fraction	P_2 fraction
Control		390.5	377.5	1000
Dopamine	100	735.0	382.5	1507.5
Apomorphine	100	700.0	374.0	1580.3
Epinine	100	732.0	380.1	1601.5
ADTN	100	820.0	376.7	1800.0

* Values represent the mean from three separate experiments.

Table 2. Calculated inhibition constants for various drugs on dopamine-stimulated cyclic AMP production in subcellular fraction P_2 of the rabbit retina*

Drug	K_i (M)
Fluphenazine	5.5×10^{-9}
Chlorpormazine	5×10^{-8}
Thioridazine	1×10^{-7}
(+)-Butaclamol	4.5×10^{-8}
(−)-Butaclamol	$> 10^{-6}$
(α)-Fluphenthixol	1.0×10^{-9}
(β)-Fluphenthixol	$> 10^{-6}$

* Reported K_i values were calculated from the relationship $IC_{50} = K_i (I + S/K_m)$, where S is the concentration of dopamine (100 μM) and K_m is the concentration of dopamine required for half-maximal stimulation of adenylate cyclase activity (5 μM).

retina. In addition, we have demonstrated the localization of [^{3}H]dopamine uptake and release systems specifically within the amacrine (P_2) synaptosomal fraction [15]. Several investigations have shown an increase in adenylate cyclase activity in the presence of dopamine and several dopamine agonists in homogenates of retina [12–14]. Others have reported the presence of an adenylate cyclase in retina sensitive to light [20, 21]. The studies described herein identify the specific subcellular localization of the dopamine-sensitive adenylate cyclase in the P_2 fraction of retina. These data suggest that amacrine cells may stimulate adenylate cyclase systems within bipolar and/or ganglion cells by interacting with dopamine receptors which are pharmacologically similar to those previously characterized in the rat striatum [22]. The fractionation procedure for the isolation of two synaptosomal preparations is an excellent model system for the study of all aspects of the dopamine system, including uptake, release and cyclase activity, and should contribute to a better understanding of the role of dopamine and possibly the amacrine cell in synaptic transmission in retina.

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REFERENCES

1. O. Hornykiewicz, *Pharmac. Rev.* **18,** 925 (1966).
2. A. Carlsson and M. Lindquist, *Acta pharmac. tox.* **20,** 140 (1963).
3. H. Nyback and G. Sedvall, *J. Pharmac. exp. Ther.* **162,** 294 (1974).
4. J. W. Kebabian, G. L. Petzold and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **69,** 2145 (1972).
5. Y. Clement-Cormier, J. W. Kebabian, G. L. Petzold and P. Greengard *Proc. natn. Acad. Sci. U.S.A.* **71,** 1113 (1974).
6. M. Karobath and H. Leitich, *Proc. natn. Acad. Sci. U.S.A.* **69,** 2145 (1964).
7. A. S. Horn, A. Cuello and R. J. Miller, *J. Neurochem.* **22,** 265 (1974).
8. L. J. Traficante, E. Friedman, M. A. Oleshansky and S. Gershon, *Life Sci.* **19,** 1061 (1976).
9. K. Gale, A. Guidotti and E. Costa, *Science, N.Y.* **195,** 503 (1977).
10. Y. C. Clement-Cormier and G. A. Robison, *Biochem. Pharmac.* **26,** 1719 (1977).
11. J. W. Kebabian and J. M. Saavedra, *Science, N.Y.* **193,** 683 (1976).
12. J. H. Brown and M. Makman, *Proc. natn. Acad. Sci. U.S.A.* **69,** 539 (1972).
13. J. H. Brown and M. H. Makman, *J. Neurochem.* **21,** 477 (1973).
14. M.-B. Bucher and M. Schorderet, *Experientia* **30,** 694 (1974).
15. D. A. Redburn and T. N. Thomas, *Society for Neuroscience*, Vol. III, 579 (1977). Published by Society for Neuroscience, Bethesda, Maryland.
16. D. A. Redburn, *Expl Eye Res.* **25,** 265 (1977). *Neuroscince*, Vol. III, 579 (1977).
17. C. Cotman and D. A. Flansburg, *Brain Res.* **22,** 152 (1970).
18. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
19. B. Ehinger and B. Falck, *Arch. klin. Ophthal.* **178,** 295 (1969).
20. N. Miki, J. Keirns, F. Marcus and M. Bitensky *Expl Eye Res.* **18,** 281 (1974).
21. T. Hendriks, J. DePont, F. Daemen and S. L. Bonting, *Biochim. biophys. Acta* **330,** 156 (1973).
22. Y. C. Clement-Cormier, R. G. Parrish, G. L. Petzold, J. W. Kebabian and P. Greengard, *J. Neurochem.* **25,** 143 (1975).