Vol. 133, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 27, 1985 Pages 222-227

DOPAMINE RECEPTORS ON PHOTORECEPTOR MEMBRANES COUPLE TO A GTP-BINDING PROTEIN WHICH IS SENSITIVE TO BOTH PERTUSSIS AND CHOLERA TOXIN

Mark R. Brann and Carole L. Jelsema

Laboratory of Cell Biology, National Institute of Mental Health,
Bld. 36, Room 3A-17, Bethesda, MD 20892

Received September 25, 1985

Dopamine receptors with a pharmacological profile similar to D2 receptors are coenriched with rhodopsin in preparations of bovine retinal membranes. A high density of these receptors are present on photoreceptor membranes. The affinity of the agonist apomorphine for these receptors is decreased by the guanine nucleotides GTP and GppNHp. Treatment of photoreceptor membranes with pertussis or cholera toxin also decreased the affinity of apomorphine and eliminated the effect of GTP.

Dopamine is involved in photoreceptor physiology. Light stimulates the synthesis and release of dopamine (1), and dopamine containing amacrine cells are located in anatomical register with photoreceptors (2). Dopamine is involved in the autophagy of photoreceptors (3), and promotes light-adaptive photoreceptor movements via dopamine-D2 receptors (4). The D2 selective antagonist metoclopropamide depresses the A wave of the human ERG (5), implicating a role for D2 receptors in human photoreceptor physiology. Radioligand binding experiments have indicated a low density of D2 receptors in the total particulate fraction of crude retinal homogenates (6,7), and the affinity of agonists for retinal dopamine receptors is decreased by guanine nucleotides (6,7).

The inhibition of adenylate cyclase by dopamine in striatum and pituitary is mediated by D2 receptors (8,9,10). Inhibition of adenylate cyclase by these receptors is sensitive to guanine nucleotides and is prevented by pertussis but not by cholera toxin (9,10). The affinity of agonists for D2 receptors in striatal membranes is decreased by pertussis toxin (11) and guanine nucleotides (11,12). Pertussis toxin catalyzes the ADP-ribosylation of the guanine nucleotide binding proteins N_i and N_o in brain membranes (11,13,14). In photoreceptor membranes, pertussis toxin catalyzes the ADP-ribosylation of the guanine nucleotide-binding protein transducin (13,15). Transducin mediates activation of cGMP-dependent phosphodiesterase by bleached rhodopsin (13,16). Unlike N_i and N_o, transducin is also sensitive to ADP-ribosylation catalyzed by cholera toxin (13).

0006-291X/85 $1.50

To determine if dopamine-D2 receptors are present on photoreceptor (rod outer segment) membranes, we used radioligand binding techniques to examined several preparations of retinal membranes for dopamine-D2 receptors. The sensitivity of these dopamine receptors to guanine nucleotides, pertussis and cholera toxins were also examined.

MATERIALS AND METHODS

Membrane Preparations. Retinas were dissected from bovine eyes (Trueth & Sons, Baltimore, MD) and frozen on dry ice. The retinas were thawed, and the total particulate fraction was prepared by homogenization in 50 mM Tris-HCL, pH 7.4, 1 mM EDTA, 120 mM NaCl (TEN) followed by centrifugation at 40,000 x g for 10 min, the pellet was resuspended in TEN and centrifugation was repeated. Purified photoreceptor membranes (rod outer segments) were isolated essentially as described (17). Briefly, retinas were thawed and stirred for 3 min with isolation buffer,10 mM MOPS, pH 7.5, 60 mM KCl, 30 mM NaCl, 2 mM $MgCl_2$, 1 mM NaN_3, 1 mM dithiothreitol, 0.1 mM EGTA (IB) containing 45% sucrose followed by centrifugation at 27,000 x g for 20 min. The pellet (P1) was saved, and the supernatant diluted 1:1 with IB (no sucrose) and centrifugation repeated. The pellet was washed twice, and floated on 45% sucrose in IB followed by centrifugation at 45,000 x g for 45 min. The buoyant material was diluted 1:1 with IB and centrifugation repeated, the pellet was washed twice, resuspended in TEN, and stored at - 70°C. Electron microscopic examination of this preparation revealed intact rod outer segments and very small membrane fragments. Only a portion of the retinal photoreceptors could be isolated by this procedure. A less pure, but higher yield preparation of photoreceptors (crude photoreceptors) was prepared by homogenization of P1 in TEN containing 45% sucrose with centrifugation at 27,000 x g for 20 min. The pellet (P2) was saved, and the supernatant was diluted 1:1 with TEN, centrifuged as before and the resulting pellet was washed, and resuspended in TEN for storage at - 70°C. For preparation of the pigmented fraction, P2 was resuspended in TEN and stored at - 70°C. Approximately 40% of the retinal protein was recovered in the pigmented fraction, 20% in the crude and less than 5% in the purified photoreceptor membranes.

Rhodopsin measurements. Eyes were dark-adapted for 6 hrs before isolation of retinas and isolations and membrane preparations were performed under dim red light. Membranes were solubilized with 10% deoxycholate. Rhodopsin was measured as absorbance at 500, and rhodopsin content is reported at the ratio of absorbance at 280 (total protein) / absorbance at 500 (18).

Dopamine receptor measurements. Receptor assays, minor modifications of established procedures (12), were performed in a 0.5 ml volume of buffer 50 mM Tris-HCl, pH 7.4, 1 μM pargyline, 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$ containing 0.05 mg of retinal protein, the dopamine agonist (^{3}H)N-propyl-norapomorphine (NEN, 68 Ci/mmole) or the dopaminine antagonist (^{3}H)spiperone (NEN, 24 Ci/mmole) and cold ligands. Tissue was incubated for 30 min at 30°C, and nonspecific binding was defined by displacement with 10 μM (-)sulpiride a D2-specific antagonist. Assays were terminated by rapid filtration through GF/C filters, filters were washed with 10 ml 20 mM Tris-HCl, pH 7.4 and counted at an efficiency of 50%. Data were analyzed by nonlinear regression using a form of the Hill equation. Protein was measured by the method of Lowry *et. al.* (19).

ADP-ribosylation. Pertussis and cholera toxins were activated by incubation in 50 mM glycine, pH 8.0, 20 mM dithiothreitol, 1 mg/ml ovalbumin, for 10 min at 30 °C. Crude photoreceptor membranes were incubated with activated toxin (2.5 μg/ml), 50 mM

potassium phosphate, 20 mM thymidine, 40 μM NAD, 1 mM ATP, 1 mM GTP for 45 min at 30°C. Membranes were centrifuged at 40,000 x g for 20 min and washed once and resuspended in the buffer used in the binding assays. Control membranes were treated in an identical fashion except no toxin was included in the incubation. (^{32}P)ADP-ribosylations were performed under identical conditions with (^{32}P)-labeled NAD (NEN, 1 μ Ci/assay) as a substrate, reactions were terminated by centrifugation and samples were subsequently prepared for SDS-PAGE (20).

RESULTS AND DISCUSSION

As illustrated in Table 1, pharmacological characterization of (^{3}H)spiperone-labeled sites in both crude and purified photoreceptor membranes (rod outer segments) suggests labeling of D2-like sites (21). The dopamine agonist apomorphine showed high affinity, and this affinity was decreased by GTP and GppNHp. The dopamine antagonists sulpiride and butaclamol showed the expected stereoselectivity and relative potencies, and both the α-1 antagonist prazocin, and the serotonin antagonist, mianserin, had very low affinity for these sites (Table 1).

A high density of dopamine receptors is present in photoreceptor membrane preparations of the retina (Table 2). The density of dopamine receptors was 2-fold higher

TABLE 1

PHARMACOLOGICAL CHARACTERIZATION OF SITES LABELED WITH (^{3}H)SPIPERONE IN PHOTORECEPTOR MEMBRANES

	IC_{50} (nM)	
	Purified Photoreceptor membranes	Crude Photoreceptor membranes
apomorphine	4	3
apomorphine + GTP	19	17
apomorphine + GppNHp	15	10
(+) butaclamol	33	44
(-) butaclamol	> 10,000	> 10,000
(-) sulpiride	330	410
(+) sulpiride	6,200	7,200
prazocin	> 10,000	> 10,000

Values represent the mean of 2-3 independent experiments performed in triplicate with 6 concentrations of the indicated agents and 0.4 nM (^{3}H)spiperone. The concentrations of GTP and GppNHp were 100 μM.

TABLE 2

DOPAMINE RECEPTORS LABELED WITH (^{3}H)SPIPERONE AND (^{3}H)N-PROPYL-NORAPOMORPHINE IN PREPARATIONS OF RETINAL MEMBRANES

	(^{3}H)Spiperone		(^{3}H)NPA		Rhodopsin
	K_D	B_{max}	K_D	B_{max}	Abs 280/500
total particulate	0.4	183	0.5	210	
pigmented membranes	0.6	60	0.3	64	ND
crude photoreceptor membranes	0.4	380	0.5	430	4.2
photoreceptor membranes	0.5	710	0.6	710	2.7

K_D (nM) and B_{max} (fmole/mg protein) values were obtained from a single retinal isolation and are representative of several experiments. These values were estimated as described in methods. Assays were performed in duplicate with 6 concentrations of the indicated ligand, and nonspecific binding was defined with 10 μM (-) sulpiride. Rhodopsin content was estimated in dark-adapted retinal tissue as described in methods.

in purified photoreceptor membranes relative to crude photoreceptor membranes, while the pigmented fraction had a very low density of receptors. The rhodopsin content of the purified photoreceptor membranes was also 2-fold higher than the crude preparation, and rhodopsin was not detectable in the pigmented fraction. Dopamine receptors were 4-fold higher in the pure photoreceptor membranes than in the total particulate fraction of a total retinal homogenate. The dopaminergic agonist (^{3}H)N-propyl-norapomorphine labeled approximately the same number of binding sites as (^{3}H)spiperone in each of these membrane preparations. Since photoreceptors account for approximately 20% of the protein in the total particulate fraction of crude retinal homogneates, these data are consistent with the majority of retinal dopamine-D2 receptors being present on photoreceptor membranes.

In control photoreceptor membranes, the apomorphine-inhibition-curve was shallow with a low Hill number, suggesting receptor cooperativity or heterogeneity (Table 3, Figure 1). GTP decreased the affinity of apomorphine and increased the Hill number to unity, suggesting a homogeneous receptor population. Pertussis and cholera toxin decreased the affinity of apomorphine for dopamine receptors, and GTP was without effect

TABLE 3

AFFINITY OF APOMORPHINE FOR DOPAMINE RECEPTORS IN PHOTORECEPTOR MEMBRANES TREATED WITH TOXINS

	IC_{50} (nM)			
	- GTP		+ GTP	
control	1.9 ± 0.2	(.49 ± .06)	18.9 ± 9.5	(.98 ± .18)
pertussis toxin	6.3 ± 2.0	(.73 ± .14)	9.50 ± 2.1	(.67 ± .09)
cholera toxin	6.8 ± 2.5	(.72 ± .16)	6.40 ± 2.2	(.60 ± .15)

Values represent the mean IC_{50} ± SD (Hill number ± SD) of 3 separate toxin experiments, each experiment was performed in triplicate with 6 concentrations (1 - 300 nM) of apomorphine and 0.4 nM (^{3}H)spiperone. No effect of toxins or GTP was observed on the binding of (^{3}H)spiperone in the absence of apomorphine. Data was analyzed by a nonlinear regression to a form of the Hill equation as described in methods.

on either apomorphine-affinity or Hill number in toxin-treated membranes. Under these conditions, pertussis toxin catalyzed the ADP-ribosylation of a single protein with a Mr 40,000 in both crude and purified photoreceptor membranes (data not shown).

The colocalization of dopamine receptors and rhodopsin to photoreceptor membranes, and their coupling to a pertussis and cholera toxin sensitive GTP-binding

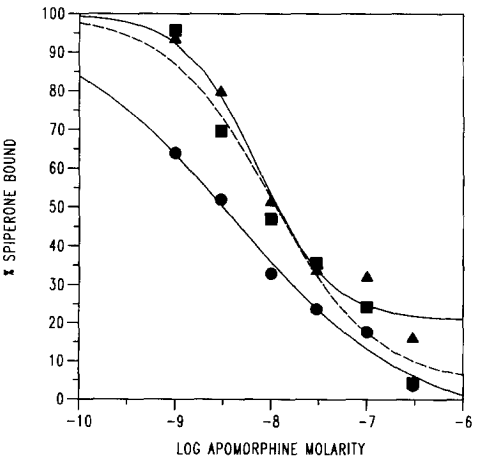

Fig. 1. Pertussis and cholera toxins decrease the affinity of apomorphine for dopamine receptors on photoreceptor membranes. Values, calculated by nonlinear regression of the Hill equation as described in methods, represent the means of triplicate determinations performed in 3 independent experiments. At all but the highest concentration of apomorphine, treatment with cholera (▲) and pertussis (■) toxin decreased the ability of apomorphine to inhibit (^{3}H)spiperone binding to dopamine receptors. Photoreceptor membranes were treated with activated toxins as described in methods.

protein, support the concept that both receptors couple to transducin, since transducin is present in a high concentration in photoreceptor membranes and has a Mr 40,000 subunit with is a substrate for ADP-ribosylation catalyzed by both toxins (13). Since D2 receptors couple to N_i in other tissues, these data indicate that coupling is tissue dependent. Together with the observation that rhodopsin is able to couple to both Ni and transducin in reconstitution systems (16,22), these data suggest a structural homology between dopamine receptors and rhodopsin, and is consistent with the known structural homology between transducin N_i and N_o (13,16,22).

ACKNOWLEDGEMENTS

The authors would like to thank Drs. J. Axelrod, M. Brownstein, G. Chader, A. Gilman, J. Kebabian, W. Klee, A. Mariani and R. Nelson for helpful suggestions and discussions and A. Dearry and M. Jaffe for sharing their data before publication. M. R. B. is a recipient of a PRAT fellowship from NIGMS, and C. L. J. is a recipient of an Eli Lilly grant.

REFERENCES

1. Iuvone, P.M., Galli, C.L., Garrison-Gund, C.K., and Neff, N.H. (1978) Science 202, 901-902.
2. Mariani, A.P., Kolb, H., and Nelson, R. (1984) Brain Res. 322, 1-7.
3. Wirz-Justice, A., Prada, M., and Reme, C. (1984) Neurosci. Lett. 45, 21-25.
4. Dearry, A., and Burnside, B.J. (1985) J. Neurochem. 44, 1753-1763.
5. Jaffe, M.J., Livingson, P., de Monsterio, F.M., and Adams, B. (1985) ARVO Abs. # 61.
6. Watling, K.J., and Iversen, L.L. (1981) J. Neurochem. 37, 1130-1143.
7. Makman, M., Dvorkin, J., and Klein, P. (1982) Proc. Natl. Acad. Sci. 79, 4212-4216.
8. Kebabian, J.W., and Caln, D.B. (1979) Nature 277, 93-96.
9. Cote, T.E., Frey, E.A., and Sekura, R.D. (1984) J. Biol. Chem. 259, 8693-8698.
10. Weiss, S., Sebben, M., Garcia-Sainz, J.A., and Bockaert, J. (1985) Mol. Pharm. 27, 595-599.
11. Kuno, T., Shirakawa, O., and Tauaka, C. (1983) Biochem. Biophys. Rec. Comm. 115, 325-330.
12. Sokoloff, P., Redouane, K., Brann, M.R., Martres, M-P., and Schwartz, J-C. (1985) N-S. Arch. Pharm. 329, 236-243.
13. Gilman, A. (1984) Cell 36, 577-579.
14. Milligan, G., and Klee, W.A. (1985) J. Biol. Chem. 260, 2057-2063.
15. Tsai, S-C., Adamik, R., Kanaho, Y., Hewlett, E.L., and Moss, J. (1984) J. Biol. Chem. 259, 15320-15323.
16. Tyminski, P.N., and O'Brien, D. F. (1984) Biochem. 23, 3986-3993.
17. Fung, B.K.K., and Stryer, L. (1980) Proc. Nat. Acad. Sci. 77, 2500-2504.
18. McConnell, D.G. (1965) J. Biol. Chem. 27, 459-473.
19. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
20. Bourne, H., and Stryer, L. (1982) J. Biol. Chem. 257, 10540-10545.
21. Seeman , P. (1980) Pharm. Rev. 32, 229-313.
22. Kanaho, Y., Tsai, S-C., Adamik, R., Hewlett, E.L., Moss, J., and Vaughan, M. (1984) J. Biol. Chem. 2259, 7378-7381.