Genet. Res. Camb. (1983), **42**, *pp.* 159–168
With 1 text-figure
Printed in Great Britain

Genetic variation in β-adrenergic receptors in mice:

A magnesium effect determined by a single gene

By JASNA MARKOVAC AND ROBERT P. ERICKSON

Department of Human Genetics, Box 015, University of Michigan Medical School, 1137 E. Catherine Street, Ann Arbor, Michigan 48109

(*Received 6 April 1983 and in revised form 19 May 1983*)

SUMMARY

Genetic variation in the amount of binding of dihydroalprenolol (a potent antagonist) to hepatocyte β-adrenergic receptors has been observed among inbred strains of mice. This variation is attributed to a differential effect of magnesium on the receptors between the high and low binding strains. Evidence for a single gene controlling the magnesium effect on dihydroalprenolol binding to β-adrenergic receptors was found using recombinant inbred lines between the high and low strains. We suggest the provisional gene symbol *Badm*.

1. INTRODUCTION

Variation has been found in numerous eukaryotic endocrine systems (Shire, 1979), both at the level of hormone synthesis (Ciaranello, 1979) and in response. Many hormones and neurotransmitters, including the catecholamines, interact with cell membrane receptors to activate adenylate cyclase and subsequently raise intracellular cyclic AMP production (Robison *et al.* 1971; Drummond *et al.* 1975). Several lines of evidence indicate that intracellular levels of cyclic AMP in various tissues are subject to genetic variation (Meruelo & Edidin, 1974; Erickson *et al.* 1979; Hindin & Erickson, 1979). Since the production of cyclic AMP by adenylate cyclase involves the interaction of several components (Katada & Ui, 1982), including regulatory units (Cassel & Selinger, 1978; Limbird *et al.* 1980; Rodbell, 1980), differences in the receptor, the guanine regulatory protein, or adenylate cyclase itself could be the source of variation ultimately seen in intracellular cyclic AMP levels.

Lafuse, Meruelo & Edidin (1979) and Lafuse & Edidin (1980) investigated these alternatives in light of an association they observed between glucagon-simulated mouse liver cAMP levels and histocompatibility (*H-2*) haplotype. Different *H-2* haplotypes showed similar basal and fluoride-stimulated adenylate cyclase activity. Futhermore, they found that the cAMP differences were not due to differences in activation by GMP-PNP, a synthetic analog of GTP which acts directly on the G protein (Londos *et al.* 1974; Lefkowitz, 1974). *H-2*-associated differences were found, however, in the capacity of glucagon to stimulate cAMP production. Scatchard analyses of the receptors from the 'high cAMP' and 'low cAMP' strains

https://doi.org/10.1017/S0016672300021625 Published online by Cambridge University Press

(Lafuse & Edidin, 1980) suggested affinity differences or possible site-site interactions (Limbird & Lefkowitz, 1976).

Our investigations of β-adrenergic binding to mouse hepatocyte membranes in the presence and absence of magnesium provide evidence for the importance of the ionic microenvironment in receptor-mediated response (Williams & Lefkowitz, 1978; Levitzki, 1981; Dibner, Wolfe & Insel, 1981; Heideman, Wierman & Storm, 1982). Furthermore, our results indicate that the apparent magnesium-induced differences in the β-adrenergic binding between the A/J and C57BL/6J mice are controlled by a single gene.

2. MATERIALS AND METHODS

(i) *Mice*

The A/J and C57BL/6J mice used were derived from mating pairs obtained from The Jackson Laboratory, Bar Harbor, Maine. All the animals used were males, between 2 and 6 months of age. The recombinant inbred lines between the A/J and C57BL/6J were maintained by brother–sister matings of the original stocks (a gift from Dr Muriel Nesbitt, University of California–San Diego, La Jolla, CA). Eight of the original 50 RI lines were tested, both AB and BA types.

(ii) *Preparation of membranes*

Livers were rapidly excised from cervically dislocated animals and minced in ice-cold 1 mM-$NaHCO_3$ buffer (pH 7·4). The suspension was homogenized with a Thomas Model 45 tissue grinder at medium speed for 30 sec, filtered twice through cheesecloth and centrifuged at 1000 ***g*** for 10 min. The pellet was resuspended in 1 mM-$NaHCO_3$ buffer (pH 7·4), homogenized for 15 sec at medium speed, and recentrifuged at 1500 ***g*** for 15 min. The pellets were quick-frozen in a dry-ice ethanol bath and stored at −20 °C.

(iii) *β-Adrenergic binding assay*

β-Adrenergic receptor binding was measured by a competitive radioligand binding assay with the potent β-adrenergic antagonist, dihydroalprenolol (DHA). Non-specific binding was determined using the antagonist, propranolol. The reaction was carried out in a total volume of 500 μl, containing 400 μl membrane suspension (0·2–0·5 mg protein), 10 nM [^{3}H]DHA (unless otherwise noted) and either 10 μM (DL) propranolol or an equivalent amount of buffer (Tris or 50 mM-K_2HPO_4, 4 mM-$MgSO_4$, as indicated). The assay was incubated for 5 min at 37 °C and terminated by addition of ice-cold buffer and emptying the sample over a Whatman GF/C filter. The filters were washed thoroughly with buffer, dried, and counted in Instagel scintillation fluid. Protein content was determined by the method of Lowry. The specific binding, expressed as fmoles/μg protein, was calculated as the difference between total binding ([^{3}H]DHA only) and non-specific binding ([^{3}H]DHA and excess propranolol).

https://doi.org/10.1017/S0016672300021625 Published online by Cambridge University Press

(iv) *Chemical and reagents*

Dihydroalprenolol hydrochloride, L-[propyl-1,2,3-^{3}H] (30–70 Ci/mmol) was obtained from New England Nuclear. (DL)-propranolol, and Trizma HCl and base were purchased from Sigma Chemical Company. All other reagents were obtained from Mallinckrodt Chemical Works.

3. RESULTS

Variation in β-adrenergic binding of the potent β-antagonist, [^{3}H]dihydroalprenolol ([^{3}H]DHA), was found among inbred strains of mice (Table 1). In a Tris buffer system (50 mM Tris buffered saline), C57BL/6J hepatocytes bound $0{\cdot}68 \pm 0{\cdot}08$ fmol [^{3}H]DHA/μg protein while A/J mice bound $0{\cdot}22 \pm 0{\cdot}04$ fmol/μg protein ($P \leqslant 0{\cdot}01$). Saturation experiments and Scatchard analysis with these two strains using varying concentrations of labelled antagonists (0·5–40·0 nM [^{3}H]DHA) indicate an affinity difference in the β receptors of A/J and C57BL/6J hepatocytes (Fig. 1 A, C) ($K_D = 10{\cdot}18$ nM for A/J and 2·09 nM for C57BL/6J).

Malbon (1980) investigated β-adrenergic receptor number and responsiveness in hepatocytes from hypothyroid rats using a buffer containing 50 mM potassium phosphate and 4 mM-$MgSO_4$, pH 7·5. Since the number of investigations of β-adrenergic receptors on mammalian liver is small compared to those using other tissues, we tested the A/J and C57BL/6J strains for binding in this buffer system. When the radioligand binding assays were performed in the phosphate-magnesium buffer system, the A/J hepatocytes bound significantly more [^{3}H]DHA than did those from C57BL/6J mice ($1{\cdot}77 \pm 0{\cdot}16$ fmol/μg protein and $0{\cdot}75 \pm 0{\cdot}08$ fmol/μg protein, respectively, $P \leqslant 0{\cdot}01$). While the phosphate-magnesium buffer system increased [^{3}H]DHA binding in both strains compared to the Tris buffer system, this increase was not significant in the C57BL/6J mice, but was much more pronounced in the A/J strain. This is illustrated by comparing the ratios of specific binding relative to A/J in Table 1. Saturation experiments and Scatchard analysis in the phosphate-magnesium buffer suggest an increase in the affinity of the A/J β-adrenergic receptor for [^{3}H]DHA in the second buffer ($K_D = 1{\cdot}25$ nM). The C57BL/6J receptor affinity did not change significantly ($K_D = 2{\cdot}11$ nM) (Fig. 1 B, D).

In order to further investigate the alterations of the β-adrenergic receptor in these two buffers, we examined the effects of various ions individually on [^{3}H]DHA binding in both strains (Table 2). Only the presence of magnesium in the reaction buffer showed a differential effect on β-adrenergic binding in the A/J compared to the C57BL/6J hepatocytes. We then measured [^{3}H]DHA binding in both buffers with and without addition of magnesium to eliminate the effects of additional factors which could affect the β receptor. As also shown on Table 2, the presence of magnesium in either buffer system has equivalent effects on [^{3}H]DHA binding as compared to its absence.

We examined the genetics of this Mg^{2+}-dependent alteration of hepatocyte β-adrenergic binding using recombinant inbred (RI) lines between the A/J and

https://doi.org/10.1017/S0016672300021625 Published online by Cambridge University Press

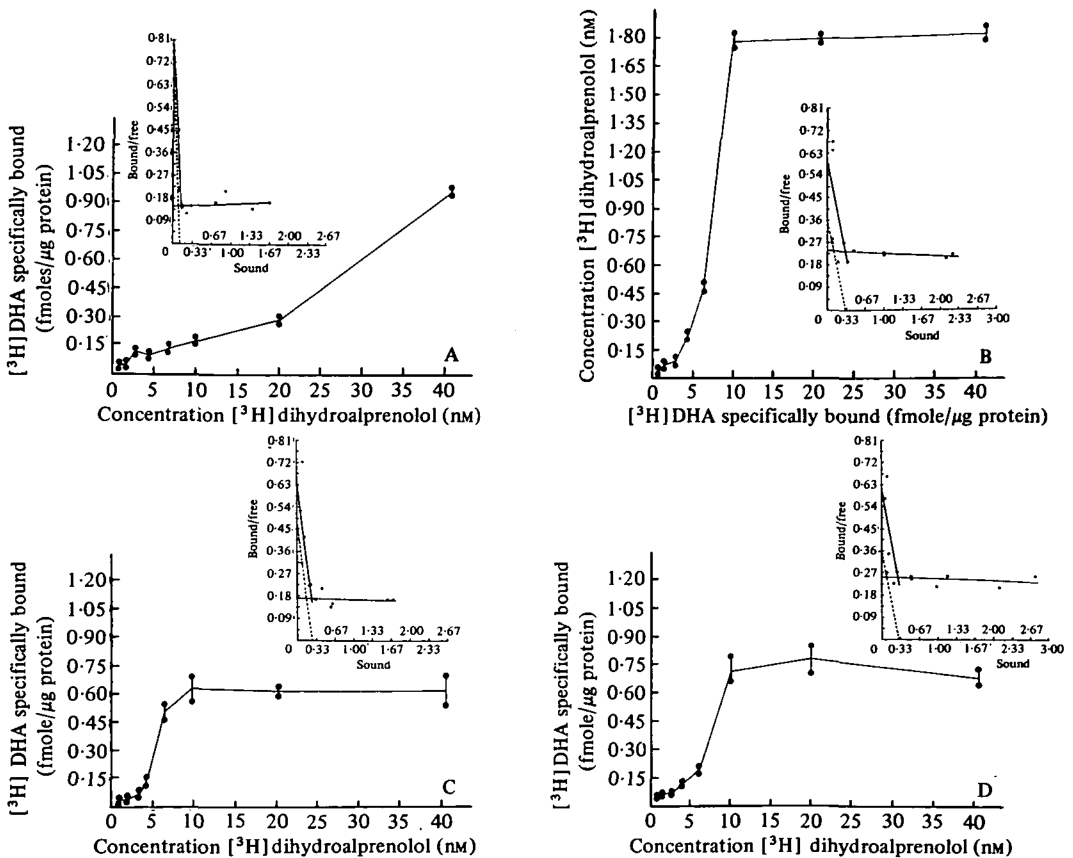

Fig. 1. Saturation plots of [³H]DHA binding to mouse hepatocyte membranes in Tris buffered saline or 50 mM-K_2HPO_4–4 mM-$MgSO_4$ buffer. Incubations were performed in the presence of varying concentrations of [³H]DHA (0·5–40·0 nM). Each point is the mean of duplicate determinations. Insets represent Scatchard analysis of saturation experiment data obtained at 25 °C. Best-fitting lines were drawn by means of a linear regression program. (A) A/J, Tris buffered saline; (B) A/J, K_2HPO_4-$MgSO_4$; (C) C57BL/6J, Tris buffered saline; (D) C57BL/6J, K_2HPO_4-$MgSO_4$.

Table 1. *Specific binding of [³H]dihydroalprenolol to liver plasma membranes*

Strain	Tris	Ratio rel. to A/J	Phosphate-Mg^{2+}	Ratio rel. to A/J	Mg^{2+} effect Ratio +Mg/−Mg
A/J	0·22 ± 0·04	1·00	1·77 ± 0·16	1·00	8·05
C57BL/6J	0·68 ± 0·08	3·09	0·75 ± 0·08	0·42	1·10

Values are the mean ± standard error for 18–20 animals.

C57BL/6J strains. In all the RI lines studied, determinations of the ratio of [³H]DHA specific binding in the presence and absence of magnesium indicate a parental segregation pattern (Table 3). The A/J mice gave a value of 8·05 for the ratio of specific binding with magnesium to without magnesium, while for the C57BL/6J strain, the ratio obtained was 1·10. The ratios for the RI lines were all

https://doi.org/10.1017/S0016672300021625 Published online by Cambridge University Press

Table 2. *Ionic composition of buffer effects on [³H]DHA binding to mouse hepatocyte membranes*

Buffer	[³H]DHA specifically bound* A/J	C57BL/6J
50 mM-K_2HPO_4 + 4 mM-$MgSO_4$	1·77 ± 0·16	0·75 ± 0·08
50 mM-Na_2HPO_4 + 4 mM-$MgSO_4$	1·76 ± 0·02	0·68 ± 0·09
50 mM-K_2HPO_4	0·25 ± 0·10	0·63 ± 0·17
50 mM-Na_2HPO_4	0·16 ± 0·03	0·70 ± 0·15
4 mM-$MgSO_4$	1·65 ± 0·09	0·74 ± 0·12
4 mM-$MgCl_2$	1·70 ± 0·12	0·69 ± 0·11
4 mM-$MnCl_2$	0·09 ± 0·02	0·56 ± 0·08
4 mM-$CaCl_2$	0·21 ± 0·04	0·69 ± 0·18
4 mM-$CaCl_2$ + 4 mM-$MgSo_4^2$	1·69 ± 0·10	0·73 ± 0·09
50 mM-Tris	0·22 ± 0·04	0·68 ± 0·08
50 mM-Tris + 4 mM-$MgSO_4$	1·65 ± 0·10	0·71 ± 0·09

* Expressed as fmol/μg protein ± standard error, $n = 3$.

Table 3. *Specific binding of [³H]dihydroalprenolol to liver plasma membranes in RI lines between A/J and C57BL/6J mice*

Strain	$-Mg^{2+}$	$+Mg^{2+}$	Ratio $+Mg^{2+}/-Mg^{2+}$
A/J	0·22 ± 0·04	1·77 ± 0·16	8·05
C57BL/6J	0·68 ± 0·08	0·75 ± 0·08	1·10
AB2	0·11 ± 0·02	0·77 ± 0·01	7·00
AB9	0·26 ± 0·04	0·56 ± 0·07	2·20
AB13	0·08 ± 0·01	0·80 ± 0·03	10·00
AB17	0·32 ± 0·10	0·52 ± 0·01	1·63
BA1	0·06 ± 0·01	0·46 ± 0·01	7·67
BA6	0·31 ± 0·01	0·58 ± 0·03	1·87
BA11	0·40 ± 0·03	0·64 ± 0·01	1·60
BA14	0·16 ± 0·04	1·28 ± 0·01	8·26

The values are the mean ± standard error for four animals.

less than 2·20 like the C57BL/6 parent (AB9, AB17, BA6, BA11) or greater than 7·00 like the A/J parent (AB2, AB13, BA1, BA14). While all the ratios could clearly be classified as A/J-like or C57BL/6J-like, a certain amount of variability was found within each group, both with respect to the ratios and actual values of [³H]DHA specific binding. Most of the RI lines did, in fact, show less specific binding both with and without magnesium than did the parent with the comparable ratio.

4. DISCUSSION

Variation in the binding of [³H]dihydroalprenolol to hepatocyte β-adrenergic receptors was found among inbred strains of mice. In a TRIS buffer system, A/J was the low binding strain, while C57BL/6J was high. Scatchard analysis of these

https://doi.org/10.1017/S0016672300021625 Published online by Cambridge University Press

two strains at varying concentrations of labelled ligand suggest an affinity difference in their β-adrenergic receptors, as indicated by the difference in K_D. In a buffer containing 50 mM potassium phosphate and 4 mM-$MgSo_4$, however, the rank order was reversed. A/J was the high binding strain, while C57BL/6J was low. Scatchard plots, under these conditions, indicate an increase in the affinity of the A/J β-adrenergic receptor for [^{3}H]DHA compared to that in the TRIS buffer system. The affinity of the β receptor on the C57BL/6J hepatocytes for the ligand did not show a significant alteration. These results support the idea that the ionic environment exerts a profound effect on ligand binding to the β-adrenergic receptor (Birnbaumer, Pohl & Rodbell, 1969; Drummond & Duncan, 1970; Drummond *et al.* 1971; Severson, Drummond & Sulakhe, 1972; Alvarez & Bruno, 1977; Williams, Mullikin & Lefkowitz, 1978; Krall & Korrenmann, 1979). Furthermore, these ion-induced changes are expressed differently on the A/J and C57BL/6J hepatocyte membranes.

Saturation plots using these strains suggested positive co-operativity in three out of the four cases. Only the A/J hepatocyte membranes in the TRIS buffer system did not show positive co-operativity nor saturability at the concentrations of [^{3}H]DHA used, presumably due to the effects exerted by the buffer on or near the β-adrenergic receptor. Positive co-operativity of [^{3}H]DHA binding has been observed in intact cells of chick, rat, and mouse embryo brain (Maderspach & Fayszi, 1982). While our preparations consisted mainly of crude membrane extracts, the presence of intact cells could contribute to the apparent positive co-operativity suggested by our saturation curves. Our inability to obtain linear Hill plots with our data (plots not shown) suggests that this phenomenon plays a minor role in the binding kinetics of our system.

In order to elucidate the nature of these ionic changes on [^{3}H]DHA binding to the hepatocyte β-adrenergic receptor, we studied the individual effects of various ions in both strains. Our results indicate that while many ions influence β-adrenergic binding, only magnesium shows different effects in the two strains by inducing apparent affinity changes.

Magnesium has previously been reported to affect binding of various hormones and neurotransmitters to their respective receptors (Chang, Blanchard & Cuatrecasas, 1983; Williams *et al.* 1978). Catecholamine binding has been reported to be magnesium dependent in several tissues, including S49 lymphoma cells (Bird & Maguire, 1978) and rabbit luteal membranes (Abramowitz, Iyengar & Birnbaumer, 1982). Although these studies report the magnesium dependence to be agonist specific and presumably involve interaction with the guanine nucleotide regulatory component of adenylate cyclase (Iyengar, 1981; Abramowitz *et al.* 1982), our results indicate a magnesium involvement in A/J mice in the binding of an antagonist, dihydroalprenolol. The cause of this discrepancy might well lie in species-, strain- and tissue-specific differences.

Our investigations were performed on mouse hepatocyte crude membrane extracts. To our knowledge, β-adrenergic binding or related magnesium effects have not been studied in this system. Fundamental differences have been reported between tissues in catecholamine binding and associated phenomena. Turkey erythrocytes, widely used for the study of the β-adrenergic system, show no effect

https://doi.org/10.1017/S0016672300021625 Published online by Cambridge University Press

of GTP on agonist binding (Bilezikian, Dornfeld & Gammon, 1978) and no desensitization (Hanski & Levitzki, 1978). Both of these have been reported in many other tissues (review: Williams & Lefkowitz, 1978). Intact human lymphocytes do not appear to require magnesium for β-adrenergic agonist stimulation of adenylate cyclase (Hui *et al.* 1981), while, as discussed above, many other systems do. The magnesium effects we find in A/J but not in C57BL/6J are presumably related to the strain differences in binding kinetic observed by Scatchard analysis. Since the alteration is concerned with antagonist binding, the magnesium effect is apparently directed at or near the level of the receptor itself, rather than at some other component of the adenylate cyclase system, such as the G protein.

We investigated the genetics of these magnesium induced differences in DHA binding by examining the recombinant inbred (RI) lines between the A/J and C57BL/6J strains. RI lines provide a powerful tool for the study of the genetics of biochemical variables (Bailey, 1971; Swank & Bailey, 1973). Since each RI line provides a number of genetically identical homozygous individuals representing a recombination of parental alleles, it is possible to examine the segregation of variables that require more than one animal for study. As our investigations of the effects of magnesium on β-adrenergic binding indicate a difference in response between the A/J and C57BL/6J strains, we employed RI lines between them to determine whether the magnesium effect segregates as a single gene. All the RI lines studied showed a parental segregation pattern of ratios of [^{3}H]DHA binding in the presence and absence of magnesium. All the ratios could clearly be classified as A/J-like or C57BL/6J-like. However, some variability was found within each group, both with respect to the actual binding values and to the ratios. This variation indicates that additional factors may be involved in the magnesium effect on the β-adrenergic binding of [^{3}H]DHA on hepatocyte membranes. Thus, our results obtained from RI lines suggest that, while other variables are probably involved, the primary effect of magnesium on β-adrenergic binding appears to be under the control of a single gene. We have provisionally designated this gene *Badm*. The strain distribution pattern for *Badm* was compared to those known for other loci but no correlation was found.

The magneisum effect, as well as being directed at the receptor itself, could alternatively involve the phospholipid matrix surrounding the receptor. In a variety of cell types, it has been shown that methylation and translocation of membrane phospholipids play an important role in the transmission of hormone mediated (Hirata & Axelrod, 1980) and differentiative (Zelenka, Beebe & Feagans, 1982) signals. This process involves two methyltransferases which ultimately convert phosphatidylethanolamine to phosphatidylcholine. Transmethylation, specifically methyltransferase I which converts phosphatidylethanolamine to phosphatidylmonomethylethanolamine, has an absolute requirement for magnesium. Furthermore, phospholipid methylation has been shown to increase membrane fluidity and β-adrenergic binding, possibly through coupling with the adenylate cyclase unit and/or the G protein (Hirata & Axelrod, 1980). Again, while this interaction of transmembrane methylation with the β-adrenergic receptor is reportedly agonist specific, antagonist binding may also be affected in certain species, strains and/or tissues. This possibility must be considered, as the

https://doi.org/10.1017/S0016672300021625 Published online by Cambridge University Press

role of an antagonist-bound receptor with respect to the G protein and cyclase unit is not yet clearly understood.

The variation we report in magnesium-induced alteration of β-adrenergic binding among inbred strains of mice allows the pursuit of these differences in terms of both the receptor and transmembrane methylation. Investigation of these components in the two strains could be used to elucidate the phenomenon of antagonist binding.

We thank Dr Muriel Nesbitt for supplying recombinant inbred mice and Mrs Rena Jones for excellent secretarial assistance. This work was partially supported by NIH Grant GM 27028.

Note added in proof

Four additional RI lines tested continue to indicate parental segregation of ratio of DHA binding in the presence and absence of Mg^{++} (AB4:8.13; AB7:2.01; BA13:9.26; BA15:9.51).

REFERENCES

ABRAMOWITZ, J., IYENGAR, R. & BIRNBAUMER, L. (1982). Guanine nucleotide and magnesium ion regulation of the interaction of gonadotropic and β-adrenergic receptors with their hormones: A comparative study using a single membrane system. *Endocrinology* **110**, 336–346.

ALVAREZ, B. & BRUNO, J. J. (1977). Activation of cardiac adenylate cyclase: Hormonal modification of the magnesium ion requirement. *Proceedings of the National Academy of Sciences U.S.A.* **74**, 92–95.

BAILEY, D. W. (1971). Recombinant inbred strains – an aid to finding identity, linkage and function of histocompatibility and other genes. *Transplantation* **11**, 325–327.

BILEZIKIAN, J. P., DORNFELD, A. M. & GAMMON, D. E. (1978). Structure-binding-activity analysis of β-adrenergic amines. I. Binding to the β receptor and activation of adenylate cyclase. *Biochemical Pharmacology* **27**, 1455–1461.

BIRD, S. J. & MAGUIRE, M. E. (1978). The agonist-specific effect of magnesium ion on binding by beta-adrenergic receptors in S49 lymphoma cells. *Journal of Biological Chemistry* **253**, 8826–8834.

BIRNBAUMER, L., POHL, S. L. & RODBELL, M. (1969). Adenyl cyclase in fat cells: Properties and the effects of adrenocorticotropin and fluoride. *Journal of Biological Chemistry* **244**, 3468–3476.

CASSEL, D. & SELINGER, Z. (1978). Mechanism of adenylate cyclase activation through the β-adrenergic receptor: Catecholamine-induced displacement of bound GDP by GTP. *Proceedings of the National Academy of Sciences U.S.A.* **75**, 4155–4159.

CHANG, K., BLANCHARD, S. G. & CUATRECASAS, P. (1983). Unmasking of magnesium dependent high-affinity binding sites for [D Ala^2, D Leu^5] enkephalin after pretreatment of brain membranes with guanine nucleotides. *Proceedings of the National Academy of Sciences U.S.A.* **80**, 940–944.

CIARANELLO, E. D. (1979). Genetic regulation of the catecholamine synthesizing enzymes. In *Genetic Variation in Hormone Systems*, vol. 1 (ed. J. M. Shire), pp. 49–61. Boca Raton, Florida: CRC Press.

DIBNER, M. D., WOLFE, R. A. & INSEL, P. A. (1981). Replacement of serum with a defined medium increases β-adrenergic receptor number in cultured glioma cells. *Experimental Cell Research* **131**, 424–427.

DRUMMOND, G. I. & DUNCAN, L. (1970). Adenyl cyclase in cardiac tissue. *Journal of Biological Chemistry* **245**, 976–983.

DRUMMOND, G. I., SEVERSON, D. L. & DUNCAN, L. (1971). Adenyl cyclase: Kinetic properties and nature of fluoride and hormone stimulation. *Journal of Biological Chemistry* **246**, 4166–4173.

ERICKSON, R. P., BUTLEY, M. S., MARTIN, S. R. & BETLACH, C. J. (1979). Variation among inbred strains of mice in 3′:5′ cyclic adenosine monophosphate levels in spermatozoa. *Genetical Research* **33**, 129–136.

https://doi.org/10.1017/S0016672300021625 Published online by Cambridge University Press

Hanski, E. & Levitzki, A. (1978). The absence of desensitization on the β-adrenergic receptors of turkey reticulocytes and erythrocytes and its possible origin. *Life Sciences* **22**, 53–60.
Heideman, W., Wierman, B. M. & Storm, D. R. (1982). GTP is not required for calmodulin stimulation of brain adenylate cyclase. *Proceedings of the National Academy of Sciences U.S.A.* **79**, 1462–1465.
Hindin, D. & Erickson, R. P. (1979). Regional and strain variation in brain 3′:5′ cyclic adenosine monophosphate of inbred mice. *Experientia* **35**, 1025–1026.
Hirata, F. & Axelrod, J. (1980). Phospholipid methylation and biological signal transmission. *Science* **209**, 1082–1090.
Hui, K. K., Kittrelle, J., Wolfe, R. N., Borst, S. & Conolly, M. E. (1981). Extracellular magnesium is not required for β-adrenergic drug-receptor interactions in intact human lymphocytes. *Life Sciences* **29**, 523–530.
Iyengar, R. (1981). Hysteretic activation of adenylyl cyclases. II. Mg ion regulation of the activation of the regulatory component as analyzed by reconstitution. *Journal of Biological Chemistry* **256**, 11042–11050.
Katada, T. & Ui, M. (1982). Direct modification of the membrane adenylate cyclase system by islet-activating protein due to ADP-ribosylation of a membrane protein. *Proceedings of the National Academy of Sciences U.S.A.* **79**, 3129–3133.
Krall, J. T. & Korenmann, S. G. (1979). Regulation of uterine smooth muscle cell β-adrenergic catecholamine-sensitive adenyl cyclase by Mg^{++} and guanylyl nucleotide. *Bioichemical Pharmacology* **28**, 2771–2775.
Lafuse, W. & Edidin, M. (1980). Influence of the mouse major histocompatibility complex, *H-2*, on liver adenylate cyclase activity and on glucagon binding to liver cell membranes. *Biochemistry* **19**, 49–54.
Lafuse, W., Meruelo, D. & Edidin, M. (1979). The genetic control of liver cAMP levels in mice. *Immunogenetics* **9**, 57–65.
Lefkowitz, R. J. (1974). Stimulation of catecholamine-sensitive adenylate cyclase by 5′-guanylyl-imidodiphosphate. *Journal of Biological Chemistry* **249**, 6119–6124.
Levitzki, A. (1981). The β-adrenergic receptor and its mode of coupling to adenylate cyclase. *CRC Critical Reviews in Biochemistry* **10**, 81–111.
Limbird, L. E., Gill, M. D., Stadel, J. M., Hickey, A. R. & Lefkowitz, R. J. (1980). Loss of β-adrenergic receptor – guanine nucleotide regulatory protein interactions accompanies decline in catecholamine responsiveness of adenylate cyclase in maturing rat erythrocytes. *Journal of Biological Chemistry* **255**, 1854–1861.
Limbird, R. E., Gill, M. D. & Lefkowitz, R. J. (1980). Agonist-promoted coupling of the β-adrenergic receptor with the guanine nucleotide regulatory protein of the adenylate cyclase system. *Proceedings of the National Academy of Sciences U.S.A.* **77**, 775–779.
Limbird, L. E. & Lefkowitz, R. J. (1976). Adenylate cyclase coupled β-adrenergic receptors: effect of membrane lipid-perturbing agents on receptor binding and enzyme stimulation by catecholamines. *Molecular Pharmacology* **12**, 559–567.
Londos, C., Solomon, Y., Lin, M. C., Harwood, J. P., Schramm, M., Wolf, J. & Rodbell, M. (1974). 5′-Guanylylimidodiphosphate, a potent activator of adenylate cyclase systems in eukaryotic cells. *Proceedings of the National Academy of Sciences U.S.A.* **71**, 3087–3090.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.
Maderspach, K. & Fajszi, C. (1982). β-Adrenergic receptors of brain cells: membrane integrity implies apparent positive cooperativity and higher affinity. *Biochimica et Biophysica Acta* **692**, 469–478.
Malbon, C. C. (1980). Liver cell adenylate cyclase and β-adrenergic receptors. *Journal of Biological Chemistry* **255**, 8692–8699.
Meruelo, D. & Edidin, M. (1974). Association of mouse liver adenosine 3′,5′-cyclic monophosphate (cyclic AMP) levels with histocompatibility-2 genotype. *Proceedings of the National Academy of Sciences U.S.A.* **72**, 2644–2648.
Robison, G. A., Butcher, R. W. & Sutherland, E. W. (1971). Cyclic AMP and hormone action. Chap. 2 in *Cyclic AMP*, pp. 17–47. New York: Academic Press.
Rodbell, M. (1980). The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature* **284**, 17–22.

https://doi.org/10.1017/S0016672300021625 Published online by Cambridge University Press

SEVERSON, D. L., DRUMMOND, G. I. & SULAKHE, P. V. (1972). Adenyl cyclase: Kinetic properties and hormonal stimulation. *Journal of Biological Chemistry* **247**, 2949–2958.

SHIRE, J. M. (1979). The uses and consequences of genetic variation in hormone systems. In *Genetic Variation in Hormone Systems*, vol. 1 (ed. J. M. Shire), pp. 1–9. Boca Raton, Florida: CRC Press.

SWANK, R. T. & BAILEY, D. W. (1973). Recombinant inbred lines: Value in genetic analysis of biochemical variants. *Science* **181**, 1249–1252.

WILLIAMS, L. T. & LEFKOWITZ, R. J. (1978). *Receptor Binding Studies in Adrenergic Pharmacology*. New York: Raven Press.

WILLIAMS, L. T., MULLIKIN, D. & LEFKOWITZ, R. J. (1978). Magnesium dependence of agonist binding to adenylate cyclase-coupled hormone receptors. *Journal of Biological Chemistry* **253**, 2984–2989.

ZELENKA, P. S., BEEBE, D. C. & FEAGANS, D. E. (1982). Transmethylation of phosphatidylethanolamine: an initial event in embryonic chicken lens fiber cell differentiation. *Science* **217**, 1265–1267.

https://doi.org/10.1017/S0016672300021625 Published online by Cambridge University Press