Regulation of cardiac adenylate cyclase activity in rodent models of obesity

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We have investigated β -adrenergic regulation of adenylate cyclase activity in heart tissue membranes from the genetically obese Zucker rat, the genetically obese CBA mouse and the genetically obese diabetic (*db/db*) mouse. Responsiveness to β -adrenergic stimulation was impaired in membranes from the obese Zucker rat, but not in the other models. The membranes from obese Zucker rats showed both decreased β -adrenergic-receptor number and altered coupling between β -adrenergic receptors and the stimulatory guanine-nucleotide-binding protein, G_s . In contrast, no alterations in either the levels of G_s or the functional interaction between this protein and the catalytic moiety of adenylate cyclase were observed. In these three genetic models of obesity we observe dissimilar alterations in the control of adenylate cyclase.

INTRODUCTION

Heterotrimeric guanine-nucleotide-binding regulatory proteins (G-proteins) play a pivotal role in transducing information between cell-surface receptors and signal generators [1–3]. The control of intracellular concentrations of cyclic AMP is vital for the integration of many physiological responses. Hormonal regulation of adenylate cyclase plays a pivotal role in this process, and controls on this enzyme may be either positive or negative. Receptors which activate adenylate cyclase do so via the G-protein G_s , whereas receptors which mediate inhibition of the enzyme interact with the G_i system [1–3]. Three highly homologous species called G_i -1, G_i -2 and G_i -3 [4] are known to exist, but the functional significance of ' G_i ' multiplicity remains to be fully elucidated. However, independent studies from a number of laboratories suggest that at least G_i -2 can transduce inhibitory effects on adenylate cyclase [5–7]. Changes in the responsiveness of adenylate cyclase to hormonal regulation are well documented to occur in a number of metabolic disease states (see [8] for review). These alterations are apparently tissue-specific and are thought to represent a means to alter the regulation of cellular functions by hormones.

In the control of cardiac function, cyclic AMP has a positive inotropic action, promoting cardiac contraction. Defects in the ability of catecholamines to stimulate adenylate cyclase have been observed in membranes prepared from failing versus non-failing human hearts and, although not firmly established, this may relate to the etiology of human heart failure [9,10]. Furthermore, in human obesity there is a well-established link between obesity and an increased risk of impaired cardiac functioning [11]. Because of this, it is of considerable interest to investigate potential alterations in hormonal regulation of adenylate cyclase in model systems of obesity. The obese/diabetic mouse and the obese Zucker rat both arise as the consequence of homozygous expression of a single recessive gene [12–15] and, although the genetics of obesity in CBA/Ca mice are less certain [16,17], all of these models provide systems which have been used to assess perturbations of metabolism associated with obesity.

Hearts from obese Zucker rats have been shown to have impaired contraction in response to secretin, a peptide hormone which functions via the activation of adenylate cyclase [18]. This is correlated with a decreased stimulation of cardiac membrane adenylate cyclase by secretin. Adenylate cyclase activity assessed in the presence of other R_s (receptors coupled in a stimulatory manner to adenylate cyclase)-linked hormones, such as glucagon and β -adrenergic agonists, has been shown to be decreased in membranes from obese Zucker rats [19]. However, the locus of the defect has not been determined.

The studies described herein were conducted to ascertain if the stimulation of adenylate cyclase was defective in membranes prepared from the hearts of obese *db/db* mice and obese CBA/Ca mice compared with their lean controls, and to explore the molecular basis of the known defect in β -adrenergic stimulation of adenylate cyclase in membranes from obese Zucker rats.

MATERIALS AND METHODS

Materials

ATP, BSA, (–)-isoprenaline (+)-bitartrate and NaF were from Sigma. Phosphocreatine, creatine kinase, GTP and guanosine 5'-[γ -thio]triphosphate (GTP[S]) were from Boehringer Mannheim. Radiochemicals were from Amersham International. Ro-20-1724 was kindly given by Roche, Welwyn Garden City, Herts., U.K. All other chemicals were of A.R. grade from BDH Chemicals.

Animals

Male genetically obese *db/db* diabetic, lean (+/+) mice and male lean and obese Zucker rats were obtained from Olac, Shaws Farm, Bicester, Oxon., U.K. They were allowed access *ad libitum* to standard rat chow and water. Male CBA/Ca mice were obtained from Dr. Peter V. Taberner, Department of Pharmacology, University of Bristol, U.K. The CBA/Ca mice included genetically obese diabetic animals and age-matched genetically lean normal animals as controls. The colonies were housed at 20–22 °C in cages of four mice with a 12 h light/dark

Abbreviations used: G-protein, guanine nucleotide-binding protein; G_s , the G-protein coupled to the stimulation of adenylate cyclase; G_i , the G-protein coupled to the inhibition of adenylate cyclase; GTP[S], guanosine 5'-[γ -thio]triphosphate; R_s , receptors coupled in a stimulatory manner to adenylate cyclase; $K_{0.5}$, concentration causing half-maximal effect; V_{max} , maximal specific activity; 125 I-CYP, iodinated cyanopindolol; K_d , equilibrium dissociation constant; B_{max} , maximal specific binding per unit of membrane protein.

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Fig. 1. Dose-response curves for isoprenaline stimulation of adenylyl cyclase in cardiac membranes from lean and obese *db/db* mice, CBA/Ca mice and Zucker rats

Various concentrations of isoprenaline were used to activate adenylyl cyclase in membranes prepared from hearts obtained from (a) lean (○) and obese (●) *db/db* mice, (b) lean (○) and obese (●) CBA/Ca mice and (c) lean (○) and obese (●) Zucker rats. The data are expressed as a percentage of maximal stimulation of adenylyl cyclase above that recorded in the presence of 100 μ M-GTP alone. The quantitative data for these experiments are presented in Table 1. The plot shows means $\pm$ S.D. from typical experiments which were repeated four times with separate membrane preparations for each animal model. (d) displays dose-response curves for isoprenaline activation of adenylyl cyclase in cardiac membranes of lean (○) and obese (●) Zucker rats when GTP (100 μ M) was replaced by GTP[S] (100 μ M). This experiment was performed twice with different membrane preparations. Quantitative data are provided in the text.

cycle. The mice had free access to pelleted CRM breeding diet (Labsure, Cambridge, U.K.) and water. They were killed by cervical dislocation at 7–12 weeks of age, and Zucker rats were killed at 22 weeks of age. Heart tissue membranes were prepared by the method of Chatelain *et al.* [19].

Enzymic assays

Assays for adenylyl cyclase activity were performed in 25 mM-triethanolamine/HCl, 5 mM-MgSO₄, 0.1 mM-Ro-20-1724, 0.5 mM-EDTA, 22 mM-disodium phosphocreatine, 1 mM-dithiothreitol, 1 mM-ATP and 1 unit of adenosine deaminase/ml. Incubations were carried out for 30 min, over which time the reaction rate was linear. Initial rates of reaction were measured. Adenylyl cyclase activity was assessed by using a cyclic-AMP-binding protein [20].

Receptor binding experiments

Binding of ¹²⁵I-cyanopindolol (¹²⁵I-CYP) to membranes was performed as described previously [17]. Briefly, assays (250 μ l) were performed in 20 mM-Hepes, pH 8.0, containing 5 mM-MgCl₂ and 1 mM-EDTA (buffer A) for 45 min at 30 °C, over which time equilibrium was achieved. ¹²⁵I-CYP bound to the membranes was collected by vacuum filtration through Whatman GF-C filters,

which were then washed with 4 $\times$ 4 ml of ice-cold buffer A. Non-specific binding was determined in parallel assays by inclusion of 10 μ M-isoprenaline. Non-specific binding was subtracted from total binding in order to determine specific binding.

Analysis of the specific binding data was performed with the ISI(R) software GraphPAD programme, version 2. All curves were fitted by non-linear regression analysis of data to a sigmoid curve fit unless otherwise stated.

RESULTS

The non-selective β -adrenergic agonist isoprenaline stimulated adenylyl cyclase activity in cardiac membranes from both lean (+/+) (2.7 ± 0.2 pmol/min per mg of membrane protein) and obese (*db/db*) (2.5 ± 0.2 pmol/min per mg of membrane protein) mice to similar maximal extents (Fig. 1a). Dose-response curves for isoprenaline stimulation of adenylyl cyclase in membranes from lean (+/+) ($K_{0.5} = 322$ nM) and obese (*db/db*) ($K_{0.5} = 406$ nM) mice were similar (see Table 1). Furthermore, there was no difference in the activity of adenylyl cyclase measured in the presence of GTP alone (Table 1) when membrane preparations from either lean or obese animals were used. Thus there was no decrease in either the fold stimulation or the absolute specific

Table 1. $V_{\max}$ and $K_{0.5}$ values for GTP and isoprenaline stimulation of adenylate cyclase in cardiac membranes of lean and obese *db/db* mice, CBA mice and Zucker rats

Adenylate cyclase activity was measured in cardiac membranes from lean and obese *db/db* mice, CBA/Ca mice and Zucker rats. The maximal activity measured both in the presence of GTP (100 μ M) alone and in the additional presence of maximally effective concentrations of isoprenaline are recorded, as is the $K_{0.5}$ for isoprenaline in the presence of 100 μ M-GTP (percentage errors in parentheses). Adenylate cyclase activities are presented as means $\pm$ S.D. ($n = 4$).

Model	Phenotype	Maximal adenylate cyclase activity (pmol/min per mg of membrane protein)		$K_{0.5}$ for isoprenaline (nM)
		GTP	GTP + isoprenaline	
<i>db/db</i>	Lean	1.2 ± 0.1	2.7 ± 0.2	322 (0.4)
	Obese	1.1 ± 0.1	2.5 ± 0.2	405 (0.2)
CBA/Ca	Lean	1.5 ± 0.1	3.3 ± 0.3	316 (16.5)
	Obese	1.6 ± 0.1	3.5 ± 0.4	301 (0.5)
Zucker	Lean	1.1 ± 0.1	6.4 ± 0.8	363 (0.8)
	Obese	1.0 ± 0.1	4.4 ± 0.5	867 (1.6)

Table 2. Activities of adenylate cyclase and binding parameters of 125 I-CYP in membranes prepared from the hearts of lean and obese Zucker rats

The data relating to adenylate cyclase specific activities are means $\pm$ S.D. of a typical experiment repeated four times with separate membrane preparations. The binding parameters for 125 I-CYP are derived from the data displayed in Fig. 2.

Rats	Adenylate cyclase activity (pmol/min per mg of membrane protein)		Binding of 125 I-CYP	
	Forskolin (100 μ M)	NaF (20 mM)	$B_{\max}$ (fmol/mg)	K_d (pM)
Lean	15.6 ± 1.1	2.5 ± 0.3	173 ± 7.4	50 ± 6.0
Obese	14.9 ± 1.5	2.6 ± 0.25	124 ± 6.2	48 ± 7.2

activity as regards the ability of isoprenaline to stimulate adenylate cyclase activity in cardiac membranes from the obese animals.

Similar experiments were performed on heart tissue membranes from lean and obese CBA/Ca mice. Fig. 1(b) shows the dose-response curve for isoprenaline stimulation of adenylate cyclase in membranes from lean and obese CBA/Ca mice. As with the *db/db* mice, there was no difference in adenylate cyclase activity in the presence of GTP alone (lean, 1.5 ± 0.1 pmol/min per mg of membrane protein; obese, 1.6 ± 0.1 pmol/min per mg of membrane protein), in the $V_{\max}$ at saturating concentrations of isoprenaline (lean, 3.3 ± 0.3 pmol/min per mg; obese, 3.5 ± 0.4 pmol/min per mg) or in the $K_{0.5}$ for activation by isoprenaline (lean, 316 nM; obese, 301 nM) (Table 1).

The capacity of isoprenaline to stimulate adenylate cyclase in cardiac membranes from Zucker rats was also examined (Fig. 1c). The activities in the presence of 100 μ M-GTP were similar in heart membranes derived from lean (1.1 ± 0.1 pmol/min per mg of membrane protein) and obese (1.0 ± 0.1 pmol/min per mg of membrane protein) Zucker rats, but the $V_{\max}$ values for adenylate

Fig. 2. Binding of 125 I-CYP to cardiac membranes prepared from lean (●) and obese (○) Zucker rats

The plot shows specifically bound radioligand versus total concentration of radioligand. The data show a typical experiment which was repeated three times with different membrane preparations.

cyclase activity as assessed in the presence of saturating concentrations of isoprenaline in membranes from the obese animals was some 30 % lower than that found for membranes from lean animals (lean, 6.4 ± 0.8 pmol/min per mg of membrane protein; obese 4.4 ± 0.5 pmol/min per mg of membrane protein). Furthermore, the $K_{0.5}$ value for the ability of isoprenaline to activate adenylate cyclase was higher in heart membranes from obese animals (867 nM) compared with that found for membranes from lean animals (363 nM) (Table 1).

These differences in adenylate cyclase regulation in Zucker rats were analysed further. Stimulation of adenylate cyclase activity by 100 μ M-forskolin yielded similar results in heart membrane preparations from lean and obese Zucker rats as did stimulation by NaF (Table 2). Furthermore, levels of G_s α -subunit assessed by immunoblotting with an antiserum (CS1) [22] which identifies the C-terminal decapeptide of forms of G_s α -subunit were similar in the heart membranes of lean and obese Zucker rats. Levels of G_s in obese animals were 95 ± 12 % (mean $\pm$ S.D.; $n = 4$) of those found in membranes from the lean animals.

The stimulation of adenylate cyclase by various concentrations of isoprenaline in the presence of GTP[S] in heart membranes of lean and obese Zucker rats was investigated (Fig. 1d). Utilizing membranes from lean animals, we found that under the conditions of the experiment the $K_{0.5}$ for isoprenaline stimulation of adenylate cyclase was decreased from 363 nM when GTP was utilized as the guanine nucleotide, to 32 nM. Thus the $K_{0.5}$ for isoprenaline stimulation was decreased by a factor of some 11-fold by utilizing GTP[S] rather than GTP as the guanine nucleotide. With membranes from obese animals, the $K_{0.5}$ for isoprenaline stimulation of adenylate cyclase in the presence of GTP[S] was 227 nM (Fig. 1d), some 4-fold lower than when GTP was utilized as the guanine nucleotide. Thus the decrease in $K_{0.5}$ for isoprenaline stimulation of adenylate cyclase when utilizing GTP[S] is smaller in heart membranes from obese animals than from lean animals.

The number of β -adrenergic receptors and the affinity of these receptors for the high-affinity non-selective β -adrenergic an-

Fig. 3. ^{125}I -CYP/isoprenaline competition-binding study with cardiac membranes from lean and obese Zucker rats

Various concentrations of isoprenaline were used to compete with a fixed concentration of ^{125}I -CYP (100 pM) for binding to β -adrenergic receptors in cardiac membrane preparations from lean (a) and obese (b) Zucker rats. Experiments were performed in the absence (○) and presence (●) of 50 μM -GTP[S]. The plots show means $\pm$ S.D. for a typical experiment repeated four times with separate membrane preparations. The relevant binding parameters deduced by computer fit of the data are presented in Table 3.

tagonist ^{125}I -CYP was assessed in heart membranes from both lean and obese Zucker rats (Fig. 2). Data were analysed to derive B_{max} and K_d values (Table 2). This identified a decrease by some 30% in β -adrenergic-receptor number in cardiac membranes from the obese animals compared with those found for membranes from lean animals. No difference in binding affinity of the receptors for ^{125}I -CYP was noted in the two membrane preparations (Table 2).

An analysis of the physical coupling between β -adrenergic receptors and G_s in heart membranes from lean and obese Zucker rats was performed by examining ^{125}I -CYP/isoprenaline competition studies in the presence and absence of the poorly hydrolysed GTP analogue GTP[S] (Figs. 3a and 3b). The binding parameters deduced by computer fitting of the data are presented in Table 3. By utilizing membranes from lean animals, it was found that in the absence of GTP[S] the competition curve (Fig. 3a) exhibited a Hill coefficient (h) of 0.36, indicative of a non-uniform population of agonist recognition sites among the β -adrenergic receptors. This could either indicate apparent negative

co-operativity or the presence of low- and high-affinity receptor populations. As guanine nucleotides are known to alter the affinity of agonist binding, it is possible that both G_s -coupled and -uncoupled populations exist. Indeed, consistent with this, upon addition of GTP[S] the Hill coefficient approached unity, indicating that negative co-operativity had been abolished. Thus in membranes from lean Zucker rats the population of β -adrenergic receptors can be converted from a mixed population, in which some of the β -adrenergic receptors interact with G_s and there are some free receptors, into a uniform population of free receptor and free G_s by the inclusion of GTP[S] in the assay. In this situation all of the receptors bind agonist with low-affinity state. Competition studies with heart membranes from obese animals performed in the absence of GTP[S] again showed a Hill coefficient (0.42) substantially lower than 1.0 (Fig. 3b and Table 3). In contrast with what was found with membranes from the lean animals, inclusion of GTP[S] did not produce a homogeneous population of binding sites, but rather a Hill coefficient of 0.57 was found. When the data of Fig. 3(b) were subjected to two-component competition curve-fit analysis, it was demonstrated that in heart membranes from the obese animals only 84% of the β -adrenergic receptors were in the low-affinity state for agonist in the presence of GTP[S] (Table 3).

DISCUSSION

db/db mice and obese diabetic CBA/Ca mice have similarities to type II non-insulin-dependent diabetes coupled with obesity in humans. In contrast, obese Zucker rats are not diabetic, but nevertheless are profoundly insulin-resistant, as evidenced by their clear hyperinsulinaemia.

Here we show that, although cardiac membranes from the obese Zucker rat demonstrated decreased β -adrenergic stimulation of adenylate cyclase compared with lean controls (Fig. 1c), the other two model systems showed no differences (Figs. 1a and 1b) in either efficacy or maximal effectiveness of β -adrenergic stimulation of adenylate cyclase between the obese test animals and appropriate lean controls. Thus such an assessment distinguishes the non-diabetic obese Zucker rat from the other obese models where diabetes is concurrent.

A number of molecular mechanisms could be envisaged to explain a decreased responsiveness of adenylate cyclase to β -adrenergic stimulation in obese Zucker rats. These include decreased β -adrenergic-receptor number; decreased coupling between β -adrenergic receptors and G_s ; decreased levels of G_s ; decreased response of the adenylate cyclase catalytic subunit to stimulation by G_s and increased tonic inhibitory input to adenylate cyclase from G_i . Although previous studies by others [19] have not addressed this matter, such investigations did record decreases in the stimulation of adenylate cyclase in response to secretin, glucagon and isoprenaline which were not equal in magnitude. We suggest that different responses may indicate that the central defect occurs at the level of the receptor, for it might be expected that defects at a level distal to the receptor would express themselves equally no matter which R_s receptor agonist was used to stimulate adenylate cyclase.

Here, we have shown that the functioning of the catalytic unit of adenylate cyclase and its interaction with G_s is unaffected in each of the model systems, as stimulation of adenylate cyclase by both forskolin and NaF is unaltered between the obese animals and their lean controls (Table 2). However, we have provided evidence for differences between lean and obese Zucker rats in the coupling of β -adrenergic receptors and G_s in cardiac membranes (Table 3). It has been shown previously that approx. 90% of the total β -adrenergic-receptor population in heart can be inactivated by use of the irreversible β -adrenergic antagonist

Table 3. Analysis of ^{125}I -CYP/isoprenaline competition-binding studies in cardiac membranes from lean and obese Zucker rats

Data from Figs. 3(a) and 3(b) were subjected to curve-fit analysis on both sigmoid and competition (two-component) types of curve fit. IC_{50} is the concn. giving half-maximal inhibition. K_L and K_H represent the equilibrium dissociation constant for the low-affinity site and the high-affinity site respectively. B_L and B_H represent the percentage of receptors in low-affinity and high-affinity state respectively. N/A, not applicable. The computer program will not adequately fit these data to a two-site model.

Rats	GTP[S] [absent (–) or present (+)]	Sigmoid curve fit			
		Goodness of fit (r2)	Hill coefficient	IC_{50} for isoprenaline (nM)	
Lean	–	0.984	0.36 (33.6%)	4	(7.8%)
Lean	+	0.997	1.38 (7.4%)	1202	(0.5%)
Obese	–	0.993	0.42 (18.5%)	39	(18.5%)
Obese	+	0.995	0.57 (12.8%)	2338	(3.3%)

Competition (two-component) curve fit						
		Goodness of fit (r2)	K_L (nM)	K_H (nM)	B_L	B_H
Lean	–	0.988	354 (3.8%)	4 (2.3%)	37%	63%
Lean	+	0.998	N/A	N/A	N/A	N/A
Obese	–	0.998	1290 (2.3%)	14 (1.1%)	37%	63%
Obese	+	0.995	1463 (1.1%)	5 (4.4%)	84.9%	15.1%

N-[2-hydroxy-3-(1-naphthoxy)propyl]-*N*-bromoacetylene-diamine ('NHNP-NBE'), without decreasing the maximal activation of adenylate cyclase achievable by β -adrenergic-receptor agonists [23]. This implies that there must be a large reserve of spare β -adrenergic receptors in rat heart tissue. This being true, any decrease in receptor number would have to be very large in order to decrease the V_{max} stimulation of adenylate cyclase by isoprenaline. We noted that levels of this receptor were some 30% lower in cardiac membranes from obese Zucker rats in comparison with lean controls (Table 2). Such a small decrease is unlikely to be sufficient to account for the observed decrease in V_{max} activity when isoprenaline is used to activate adenylate cyclase. We also noted that levels of G_s α -subunit polypeptide were similar in heart membranes from lean and obese Zucker rats, as assessed by immunoblotting with an antiserum which identifies the C-terminal region of forms of this G-protein. Thus we suggest that the lesion is produced at the level of coupling of receptor and G_s . In order to assess this, we took advantage of the fact that poorly hydrolysed GTP analogues are known to modify the interactions of receptors and G-proteins and that dose-response curves for agonist stimulation of adenylate cyclase differ depending on the identity of the guanine nucleotide used [24–26]. This relates to the fact that a lower occupancy of the receptor population is required to achieve half-maximal activation of adenylate cyclase when GTP[S] rather than GTP is used as nucleotide. The difference in the dose-response curves for isoprenaline stimulation of adenylate cyclase in the presence of either GTP or GTP[S] was less in membranes from the obese Zucker rats than from the lean controls. This result again indicates a difference in coupling between β -adrenergic receptors and G_s between the obese and lean Zucker rats. The transition to a population of receptors free of G_s interactions was incomplete in membranes from obese Zucker rats after addition of GTP[S] (Table 3). This implies a defect in the ability of the agonist-receptor- G_s ternary complex to dissociate to agonist-receptor and free G_s in the presence of GTP[S]. Thus a defect in the interaction of G_s and β -adrenergic receptors is apparent in heart membranes from obese Zucker rats.

It is unclear if the difference in interaction between the β -

adrenergic receptor and G_s between the lean and obese Zucker rats is produced at the level of the receptor, the G-protein or both. An alteration in receptor function could be achieved by phosphorylation of this polypeptide. It is indeed well established that phosphorylation of this receptor can occur and results in homologous desensitization [27]. On this question it is interesting to note that, since the β -adrenergic receptors' role in the signal-transduction process is primarily a catalytic one [28], in which the activation of one receptor leads to the activation of a number of molecules of G_s , modulation of β -adrenergic receptor to G_s coupling would provide a sensitive target to control responsiveness of adenylate cyclase to catecholamines. In support of the above notion, studies [9] have shown that the decreased response of membranes from failing human hearts is largely due to uncoupling of β -adrenergic receptors from G_s .

The studies reported herein support the notion that uncoupling of β -receptors is likely to be functionally significant in the impairment of catecholamine stimulation of cardiac adenylate cyclase in pathological states exhibiting poor inotropic response to catecholamines. We [29,30] and others [31] have reported previously that the stimulation of adenylate cyclase by isoprenaline and other lipolytic hormones is severely decreased in adipocytes from *db/db* mice and obese Zucker rats. No such alterations are found in the liver tissue of these animals [32,33] or in various other tissues of obese Zucker rats [19]. As such, desensitization of the adenylate cyclase system to hormonal stimulation is tissue-specific in these two obesity/type II diabetes model systems.

In the wider context of alterations in signal transduction in heart tissue of obese Zucker rats, a defect in protein kinase C signal transduction has been described [34]. The protein-kinase-C-activating phorbol esters appear to be less able to achieve the functional inactivation of glycogen synthase activity in heart tissue from the obese animals. Based on the observed 'cross-talk' between signal-transduction pathways which regulate protein kinase C and adenylate cyclase [35], studies which address these interactions are likely to shed new light on malfunctions in the hormonal regulation of cardiac tissue in obesity and heart disease.

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REFERENCES

- Birnbaumer, L., Codina, J., Mattera, R., Cerione, R. A., Hildebrandt, J. D., Sunyer, T., Rojas, F. J. & Iyengar, R. (1985) *Mol. Aspects Cell. Regul.* **4**, 131–182
- Birnbaumer, L., Abramowitz, J. & Brown, A. M. (1990) *Biochim. Biophys. Acta* **1031**, 163–224
- Gilman, A. G. (1987) *Annu. Rev. Biochem.* **56**, 615–649
- Jones, D. T. & Reed, R. R. (1987) *J. Biol. Chem.* **262**, 14241–14249
- Simonds, W. F., Goldsmith, P. K., Codina, J., Unson, C. G. & Spiegel, A. M. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7809–7813
- McKenzie, F. R. & Milligan, G. (1990) *Biochem. J.* **269**, 391–398
- Bushfield, M., Murphy, G. J., Lavan, B. E., Parker, P. J., Hruby, V. J., Milligan, G. & Houslay, M. D. (1990) *Biochem. J.* **268**, 449–457
- Houslay, M. D. (1990) in *G-Proteins as Mediators of Cellular Signalling Processes* (Houslay, M. D. & Milligan, G., eds.), pp. 197–230, Wiley and Sons, Chichester
- Bristow, M. R., Hersherberger, R. E., Port, J. D., Minobe, W. & Rasmussen, R. (1989) *Mol. Pharmacol.* **35**, 295–303
- Francis, G. S. & Cohn, J. N. (1990) *FASEB J.* **4**, 3068–3075
- Crandall, D. L. & DeGirolamo, M. (1990) *FASEB J.* **4**, 141–147
- Hummel, K. P., Dickie, M. M. & Coleman, D. L. (1966) *Science* **155**, 1127–1128
- Coleman, D. L. & Hummel, K. P. (1967) *Diabetologia* **3**, 252–256
- Ingalls, A. M., Dickie, M. M. & Snell, G. D. (1950) *J. Hered.* **41**, 317–318
- Zucker, L. M. & Zucker, T. F. (1961) *J. Hered.* **52**, 275–278
- Connelly, D. M. & Taberner, P. V. (1990) *J. Physiol. (London)* **425**, 40P
- Connelly, D. M. & Taberner, P. V. (1989) *Pharmacol. Biochem. Behav.* **34**, 255–259
- Robberecht, P., De Neef, P., Camus, J. C., Waelbroeck, M., Fontaine, J. & Christophe, J. (1983) *Pflugers Arch.* **398**, 217–220
- Chatelain, P., Robberecht, P., De Neef, P., Camus, J. C., Heuse, D. & Christophe, J. (1980) *Pflugers Arch.* **389**, 29–35
- Houslay, M. D., Metcalfe, J. C., Warren, G. B., Hesketh, T. R. & Smith, G. A. (1978) *Biochim. Biophys. Acta* **436**, 489–494
- Reference deleted
- Milligan, G. & Unson, C. G. (1989) *Biochem. J.* **260**, 837–841
- Venter, J. C. (1979) *Mol. Pharmacol.* **16**, 429–440
- Birnbaumer, L., Swartz, T. L., Abramowitz, J., Mintz, P. W. & Iyengar, R. (1980) *J. Biol. Chem.* **255**, 3542–3551
- Schramm, M. & Rodbell, M. (1975) *J. Biol. Chem.* **250**, 2232–2237
- Ross, E. M., Maguire, M. E., Sturgill, T. W., Biltonen, R. L. & Gilman, A. G. (1977) *J. Biol. Chem.* **252**, 5761–5775
- Lefkowitz, R. J., Hausdorff, W. P. & Caron, M. G. (1990) *Trends Pharmacol. Sci.* **11**, 190–194
- Levitzi, A. (1988) *Science* **241**, 800–806
- Strassheim, D., Palmer, T. & Houslay, M. D. (1991) *Biochim. Biophys. Acta* **1096**, 121–126
- Strassheim, D., Palmer, T., Milligan, G. & Houslay, M. D. (1991) *Biochem. J.* **276**, 197–202
- Vannucci, S. J., Klim, C. M., Martin, L. F. & LaNoue, K. F. (1989) *Am. J. Physiol.* **257**, E871–E878
- Palmer, T. & Houslay, M. D. (1991) *Biochim. Biophys. Acta* **1097**, 193–204
- Houslay, M. D., Gawler, D. J., Milligan, G. & Wilson, A. (1989) *Cell. Signalling* **1**, 9–22
- Van de Werve, G., Zanetti, D., Lang, U., Vallotton, M. B. & Jeanrenaud, B. (1986) *Diabetes* **36**, 310–314
- Houslay, M. D. (1991) *Eur. J. Biochem.* **195**, 9–27

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